Ionic channel changes in glaucomatous retinal ganglion cells: multicompartment modeling

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Abstract— **This research takes a step towards discovering underlying ionic channel changes in the glaucomatous ganglion cells. Glaucoma is characterized by a gradual death of retinal ganglion cells. In this paper, we propose a hypothesis that the ionic channel concentrations change during the progression of glaucoma. We use computer simulation of a multi-compartment morphologically correct model of a mouse retinal ganglion cell to verify our hypothesis. Using published experimental data, we alter the morphology of healthy ganglion cells to replicate glaucomatous cells. Our results suggest that in glaucomatous cell, the sodium channel concentration decreases in the soma by 30% and by 60% in the dendrites, calcium channel concentration decreases by 10% in all compartments, and leak channel concentration increases by 40% in the soma and by 100% in the dendrites.**

I. INTRODUCTION

Glaucoma, a chronic neurodegenerative disease of retinal ganglion cells (RGCs), is the second leading cause of irreversible blindness worldwide. It is estimated that 79.6 million of people worldwide will develop glaucoma by 2020, exacerbated by our ageing society [17]. Glaucoma is a disease of the progressive optic neuropathy which is characterized by the progressive death of RGCs leading to morphological changes in the optic nerve. The exact pathogenesis of glaucoma is not clear.

Current understanding of the ionic channels neuropathy in glaucomatous eyes is poor. Experimental data shows that calcium and sodium channel dynamics contribute to the ischemic axon injury; calcium deregulation reduces the capacity for mitochondria to buffer calcium from the cell leading to the reduced clearance of the intracellular calcium ions from the intracellular space; the entry of the extracellular calcium ions into the axoplasm plays an important role in the axon degeneration; sodium channel blockers contribute to the recovery of the optic nerve compound potentials; and that calcium channel blockers may or may not protect optic nerves from anoxia [4], [16], [21], [22].

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Histology shows that the morphology of the ganglion cells alter prior to the neurons death due to glaucoma. The ganglion cells in glaucomatous eyes have smaller soma, the structure of their dendritic tree is less complex and their dendrites are shorter compared to healthy cells [23]. Currently, it is not clear how these morphological changes affect human vision prior to cell death.

It is critically important to the future development of neuroprotective strategies for glaucoma to understand the effects of cellular alterations prior to cell death. This project aims to understand the ionic channel changes in glaucomatous ganglion cells. The results of this study may have implications for the development of tests for cellular abnormalities in human early glaucoma.

The initiation of the disease and its progression can be studied using mathematical modelling and computer simulation. In many cases, computer simulations have many advantages over *in vitro* and *in vivo* experiments and psychophysical studies, including relative simplicity to manipulate parameters and draw conclusions without the need for multiple repetitions of an experiment. In this project, we use computer simulations as a tool to study intrinsic electrophysiology of glaucomatous ganglion cells.

II. METHODS

Numerical simulations of a multicompartment model of a mouse RGC were carried out in NEURON environment and the data were analyzed in Matlab [8]. The cell's compartments representing the dendrites, soma and axon were taken as cylinders of variable diameter and length. The ionic channel concentrations varied among different compartments. The cell's morphology was taken from the NeuroMorpho database (Chalupa 189 cell) [2].

The membrane potential dynamics were simulated using Hodgkin-Huxley-type equations, similar to [6], and included sodium (I_{Na}), calcium (I_{Ca}), delayed rectifier potassium (I_{K}), A-type ($I_{\mathrm{K,A}}$), Ca-activated potassium (I_{Ca}), and leak (I_{L}), currents:

$$C_{\mathrm{m}}\frac{dV}{dt} = I_{\mathrm{Na}} + I_{\mathrm{Ca}} + I_{\mathrm{K}} + I_{\mathrm{K,A}} + I_{\mathrm{K(Ca)}} + I_{\mathrm{L}} + I_{\mathrm{stim}}, \quad (1)$$

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where V is the membrane potential, $C_{\rm m} = 1$ μF/cm^2 is the specific capacitance of the membrane, and $I_{\rm stim}$ is an intracellular stimulation current. The gating variables that describe the opening and closing of the ionic channels had dynamics as described in [6] and were modeled using the first-order kinetic equation

$$\frac{dx}{dt} = -(\alpha_x + \beta_x)x + \alpha_x,$$

where x is a gating variable, α_x and β_x are the rate constants with different dynamics for each ionic channel. All reversal potentials were fixed, except Ca^{2+} reversal potential which was varied with time according to changes in $I_{\rm Ca}$. For details refer to [6], [14].

The parameters used in simulations were as follows: potassium reversal potential $V_{\rm K} = -70$ mV, sodium reversal potential $V_{\rm Na} = 35$ mV, leak reversal potential $V_{\rm L} = -60$ mV, calcium reversal potential $V_{\rm Ca}$ is variable. Calcium dissociation constant $[{\rm Ca}^{2+}]_{\rm diss} = 10^{-6}$ M, extracellular calcium ion concentration $[{\rm Ca}^{2+}]_{\rm e} = 1.8$ mM, gas constant $R = 8.314$ J/(M·K), Faraday constant $F = 9.684 \cdot 10^4$ C/M.

Table 1: Experimental data. Summary of morpho logical changes. Average values from 64 healthy and 44 glaucomatous eyes. Adapted from [23].

	Soma area μ**m**^{2}	**Soma surface area** μ**m**^{2}	**Dendritic length** μ**m**	**Dendritic surface area** μ**m**^{2}	**Total surface area** μ**m**^{2}
Healthy	336	1,354	3,352	8,247	9,601
Glauc	276	1,104	2,381	4,294	5,398
% Diff	18	18	29	47	43

"Glauc" corresponds to glaucomatous RGCs.
"Diff"corresponds to the difference between healthy and glaucomatous cells.

First, the healthy cell morphology was altered similar to the published experimental data for glaucomatous cells [23], refer to Table 1. In particular, for every cylinder compartment representing a segment of the dendritic tree, its diameter and length was reduced by 29%. This corresponded to 47% dendritic surface area decrease, similar to experimental data. The soma length was reduced by 18%, as reported experimentally.

Second, a systematic parameter search for Na^+, Ca^{2+}, and leak conductances was carried out to find parameters that keep the cell's input resistance unchanged in glaucomatous cells, similar to the reported experimental results [23].

Last, the effects of changes in ionic channel conductances onto the intrinsic electrophysiology and passive membrane properties of the cell were investigated and compared to experimental data.

This methodology allowed us to investigate the exact ionic channel changes in the glaucomatous ganglion cells that corresponded to the cells morphological changes reported experimentally.

III. RESULTS

Changes in the morphology as described in Methods led to the altered input resistance of the cell due to the decreased surface area in glaucomatous RGCs. To keep the input resistance unchanged in the glaucomatous cell, similar to experimental data, we modified Na^+, Ca^{2+}, and leak ionic channel concentrations. A systematic parameter search showed that Na^+ channel concentration had to decrease by 30% in the soma and by 60% in the dendrites, Ca^{2+} channel concentration had to decrease by 10% in all compartments, and leak channel concentration had to increase by 40% in the soma and by 100% in the dendrites. This is summarized in Table 2. Note, increasing Ca^{2+} concentration is supported by the experimental evidence, refer to Discussions for details.

Table 2: Simulation results. Morphology and ionic channel concentrations in glaucomatous cells.

Morphology	
Dendrites diam	↓ 29%
Dendrites length	↓ 29%
Soma length	↓ 18%
Model predictions:	
Ionic channel concentrations	
Na^+	↓ 30% in soma, ↓ 60% in dend
Ca^{2+}	↑ 10% in all compartments
Leak	↑ 40% in soma, ↑ 100% in dend

These ionic channel modifications led to the changes in the passive properties of the glaucomatous RGC similar to experimental data, refer to Table 3. In the table, "RMP" corresponds to the resting membrane potential. The spike width was calculated at the half-maximum spike amplitude. $R_{\rm in}$ corresponds to the input resistance of the cell.

Note, a mouse cell was used in simulations while data for primates was presented in experiments. Since mouse cells are smaller than primate cells, their input resistance is higher ($R_{\rm in}$, column 3 in Table 3). However, the change in $R_{\rm in}$ between healthy and glaucomatous cells is similar in the experimental data and in simulations (see % Diff in column 3, Table 3).

"Spike freq" in column 6, Table 3, corresponds to the maximum spiking frequency in the experimental data and to the frequency at threshold current in the simulated experiment. Note, the simulated cell did not exhibit spontaneous frequency. Therefore, to calculate the spike frequency, a threshold current was injected into the soma in 1 pA steps until the firing started and the spiking frequency was calculated. Although our simulation values are different to the experimental values for the threshold current (compare 6.5% to 21.88% in

column 4, Table 3), the spike frequency decrease is significant (compare 7.2 Hz in the healthy cells, and 5.7 Hz in the glaucomatous cell). This implies that a glaucomatous cell needs much more energy to produce the same output frequency as a healthy cell.

Table 3: Comparison of the experimental data and simulation parameters.

	RMP	R_{in}	**Thresh hold**	**Spike width**	**Spike freq**
	mV	**MΩ**	**nA**	**ms**	**Hz**
Experiment					
Healthy	-53.03	21.73	0.31	0.43	108.1*
Glaucoma	-53.98	22.53	0.33	0.5	92.81
% Diff	1.7	3.7	6.5	25	14.9
Simulations					
Healthy	-52.41	119	0.06	1.09	7.28*
Glaucoma	-50.74	120.7	0.08	1.37	5.78
% Diff	3.2	1.43	21.88	25.36	20.58

*Note, simulated cells did not have spontaneous discharges, therefore, the spike frequency was calculated different methods in the experiments and in simulations.

Modifications in the morphology and ionic current conductances led to the increase in the spike width by 25%, decrease in the maximum spike amplitude by 10%, and decrease in the cells spiking frequency by 20%. In addition, the maximum value for the derivative of the membrane potential, dV/dt decreased and the phase plot of the impulse response changed, as illustrated in Figs. 1 and 2. These modifications did not affect the values of the resting membrane potential and input resistance significantly, similar to experimental data [23].

IV. CONCLUSIONS AND DISCUSSIONS

In this paper, using computer simulations, we explored the ionic channel concentration changes in glaucomatous ganglion cells. Using published experimental data, we constrained the model based on the RGC morphological changes in glaucoma, as reported in Table 1. We showed that to keep the input resistance of the glaucomatous ganglion cells unchanged, similar to published experimental data, the sodium, calcium, and leak conductances have to change in different proportions in the soma and in the dendrites as reported in Table 2. We validated these changes using published experimental data for the resting membrane potential, threshold current, spike width and the spike frequency, as reported in Table 3.

Our results suggest that calcium concentration has to increase in all cell compartments. This is supported by experimental data that shows that the intracellular calcium concentration is higher in glaucomatous RGCs [1], [3], [4], [15], [18]. Calcium ions play a crucial role in neuronal degeneration and calcium signalling regulates many cells functions, including synaptic plasticity and cell survival. Calcium buffering impairment has shown to be present in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, epilepsy, and schizophrenia [22].

Our modeling does not include synaptic currents. Our results suggesting that the leak channel concentration changes may be interpreted as the altered inhibitory and excitatory currents in the glaucomatous cells. This is not a surprising finding. Due to gradual death of ganglion cells in glaucoma, retinal network remodeling most likely occurs, and the bipolar and amacrine neurons that used to connect to now dead ganglion cells, form new synapses with surviving ganglion neurons.

It is left for future research to do a wider parameter search for all ionic conductances and to simulate different morphological types in the models of glaucomatous RGCs. Also, it is important to investigate the interactions between retinal neurons and Muller cells. In healthy retina, Muller cells take up excess extracellular glutamate. However, during glaucoma Muller cells cannot function optimally due to the high extracellular glutamate concentration [9], and changes such as reactive gliosis occurring [7].

It has been shown that the axons of dying glaucomatous cells are usually in a more advanced stage of degeneration than the rest of the cells [16]. Often, substances such as calcium and sodium channels blockers are used to attenuate the insult of ganglion cells axons. The most effective concentration and time of application of such substances may be investigated using the multicompartment models presented here. Specific understanding of the gradual changes in the ionic channel concentrations may help to draw conclusions and develop the most efficient therapies for specific neuropathies.

Improved knowledge of the characteristics of the cells pathology and their impact can help in the development of new methods for neuroprotection and cell rescue. The tools developed in this project may be combined with psychophysical test during various stages of glaucoma progression to understand how RGC morphological changes affect vision.

While this pilot project focuses on neural disease due to glaucoma, the tools developed here have broader applicability. They can be adapted to investigate the effects of neurons morphology changes on their intrinsic electrophysiological properties in other types of diseases, such as the striatal spiny neurons in Huntington disease and the lateral nucleus amygdale neurons in Alzheimer's disease. It has been shown that neurons go through morphological alterations in animal models of Alzheimer's and Huntington's disease [10], [11]. Studies that investigated the effect of cell morphology on the

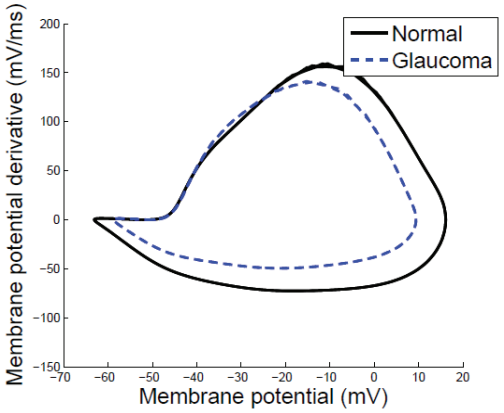

Fig. 1. A comparison of the phase plot of the impulse response for healthy and glaucomatous RGCs.

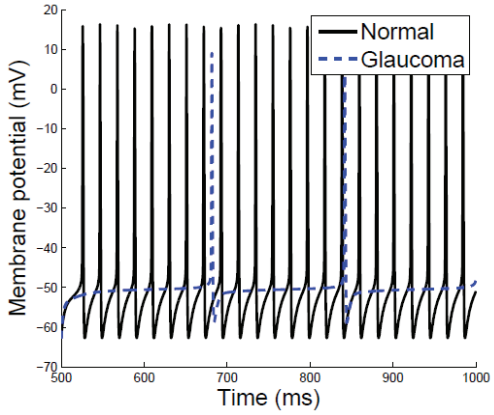

Fig. 2. A comparison of the spiking frequency for healthy and glaucomatous RGCs. A depolarizing current of 80 pA was injected into the soma.

cells electrophysiological properties in other cell types include [5], [12], [20]. Stuart and Spruston (1998) use compartmental models of neocortical pyramidal neuron dendrites to examine voltage attenuation in the cells. Using computational models the effect of neurons morphology on neurons intrinsic electrophysiology was considered in Korogod and Kulagina (1998). In this study the authors consider passive and active (Hodgkin-Huxley type) dendrites and examine the impact of the dendritic geometry on the somatopetal transfer of current.

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