Effects of Betaxolol on Hodgkin-Huxley Model of Tiger Salamander Retinal Ganglion Cell

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1. Abstract

Isolated retinal ganglion cells (RGC) elicit action potentials upon depolarization from upstream bipolar cells, which are modulated by horizontal and amacrine cells. The frequency and amplitude of action potential spikes are not only dependent on upstream input, but also the inherent intracellular and extracellular ion concentrations. The Hodgkin-Huxley model examined in this paper accounts calcium concentration changes within the cell, which has an appreciable effect due to the large changes in calcium reversal potential arising from the small intracellular calcium concentration and large extracellular calcium concentration. This paper studies the effects of a voltage gate inhibiting drug, Betaxolol, in a single cell Hodgkin-Huxley model of the tiger salamander retina. Betaxolol reduces both sodium current in an RGC, leading to reduced spiking frequency; however, calcium current is also reduced by the drug, which has an opposite effect of increasing spiking frequency. This study found that if the sodium conductance is reduced to one-third of the control value, the calcium conductance must be reduced less than one-third of its control value for the cell to have a reduced spiking frequency.

2. Introduction

2.1 Retinal Ganglion Cells

Like motor neurons, RGCs elicit action potentials upon a depolarization of the resting membrane potential to its threshold. Ion channels control the flow of charge into and out of the cell, depending on the balance between its open and closed states. The Hodgkin-Huxley model quantifies the gating dynamics of a neuron with gating variables m, n, and h. Physiologically, m and h are associated with sodium channel activation and inactivation respectively, while h is the potassium channel activation [1]. In retinal ganglion cells, three additional gating variables quantify the cell's membrane dynamics: c, a, and h_a [5]. Consequently, the gating variables affect the conductance of their respective ion species.

Unlike motor neurons, retinal ganglion cells are constantly firing action potentials [1]. The photoreceptors convert electromagnetic radiation to electrical signals through a photochemical process fostered by phosphodiesterase and rhodopsin. The strength of the response is proportional

to the amount of light absorbed by the bundle of rods and cones [3]. There are two types of RGCs; on-center and off-center. An on-center RGC depolarizes in the presence of a strong signal in its center receptive field and directly increases its firing rate. In contrast an off-center RGC hyperpolarizes in the presence of a strong signal in its center receptive field and increases its firing post-inhibition [4]. The summation of signals from upstream bipolar cells to the RGC elicits either an on, off, or on-off response [3].

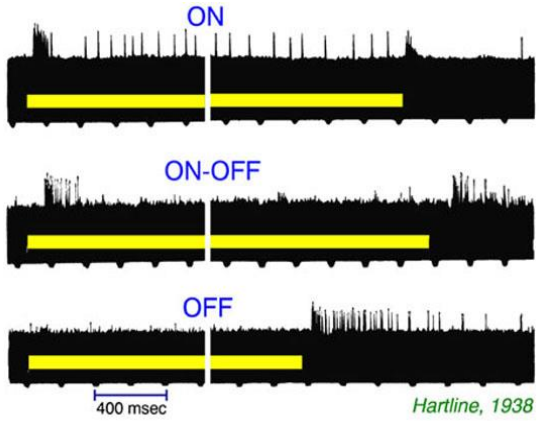

Figure 1. The on, off, or on-off response shown

2.2 Motivation

The ability to perceive light and contrast visually connects our brain with the world. Light signals are sent across the optic nerve to the brain. The phototransduction pathway converts electromagnetic radiation to electrical signals sent from photoreceptors across the different cells in the retinal layers, ending with the retinal ganglion cells. From there, the action potentials fired by the retinal ganglion cell are sent directly to the brain. In this way, understanding RGC firing dynamics could offer direct insight into how the brain processes and constructs an image.

The firing pattern of ganglion cells is the input for the visual cortex and the information for constructing images. If during the phototransduction pathway the signal is altered, then the firing frequency will change. For example, patients with diabetic retinopathy experience increased glucose levels which affect ion concentrations and conductivity [6]. At any point along the phototransduction pathway, calcium and sodium conductances may vary in the presence of retinal diseases or trauma. To gain an understanding of how varying conductances alter ganglion firing behavior, this project analyzes the effects of both calcium and sodium channel blockers, specifically betaxolol on a single RGC's firing behavior.

2.3 Approach

Since intracellular calcium concentration is exponentially lower than extracellular concentration, slight fluctuations can have diverse effects on the firing behavior of the neuron [8]. Therefore, accurately depicting differences in both sodium and calcium concentration required an empirical neuron model that accounted for changes in calcium conductance. For this project, a calcium dependent potassium channel was incorporated into the Hodgkin Huxley model according to calculations predicted by Fohlmeister [5]. A single retinal ganglion cell was modeled at varying levels of sodium and calcium conductance to qualify the effects of betaxolol on the ganglion cell's firing frequency and predict the outcome of similar drugs. Both firing frequency and amplitude were used as qualifiers for the comparison.

3. Methods

3.1 Model Comparison

The Hodgkin-Huxley model was initially created to show that the action potential of a squid axon can be can be modeled using the simple circuit model shown below.

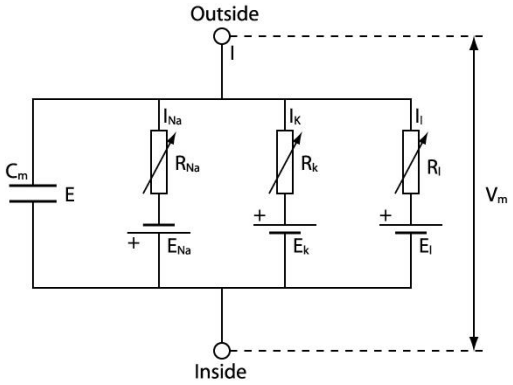

Figure 2. The circuit model of a typical Hodgkin-Huxley Neuron [9]

Fohlmeister has shown that using this basic premise of the HH model, they were able to create a model that captures the repetitive firing motion of a retinal ganglion cell in a Tiger Salamander [5]. They did this by initially observing voltage-clamp studies of the ganglion cells, and then came to the conclusion that there were five main currents to analyze. There consisted of the four voltage-gated channels – Na^+, Ca^+, non-inactivating K^+, inactivating K^+ (a type)– and one that was Ca^{2+} activated K^+ [5].

3.2 HH Parameters and Equations

The following equations were derived to model the ganglion cell of a Tiger-Salamander. [5]

$$C_m \frac{dV}{dt} = -(I_{Na} + I_K + I_{Ca} + I_a + I_{KCa}) + I_{stim} \quad (1)$$

$$I_{Na} = g_{Na} h m^3 (V - V_{Na})$$

$$I_K = g_K n^4 (V - V_k)$$

$$I_{Ca} = g_{Ca} c^3 (V - V_{Ca})$$

$$I_A = g_a a^3 h_A (V - V_k)$$

$$I_{K,Ca} = g'_{K,Ca} (V - V_k) \quad (2)$$

In this equation each I represents the current through that specific ion channel, g represents the conductances, and V represents the reversal potentials of the specific ions. The following tables are composed of all the constants we will need to solve these set of equations.

$Conductance\ (mS/cm^2)$	
g_{Na}	40.0
g_{Ca}	2.0
g_K	12.0
g_A	36.0
$g_{K,Ca}$	0.05

$Reversal\ Potentials(mV)$	
V_{Na}	$+35.0$
V_{Ca}	-75.0

$Membrane\ Conductance(\frac{uF}{cm^2})$	
C_m	1

Table 1(a), 1(b), 1(c). Variables necessary to generate impulses [5]

The following calcium conductance equation is implemented into Equation 2. The calcium dissociation constant and residual value are both shown in Equation 4.

$$g'_{KCa} = g_{KCa}\frac{([Ca]_i^2/[Ca]_{diss}^{2+})^j}{1+([Ca]_i^2/[Ca]_{diss}^{2+})^j}\ with\ j=2 \qquad (3)$$

$$[Ca]_{diss}^{2+} = 10^{-3}mM, \quad [Ca^{2+}]_{res} = 10^{-4}mM \qquad (4)$$

Due to the above equations, they were able to formulate the following equation. The tau (τ) here is important to notice as this is a parameter that can be changed to determine how the calcium is sequestered if the internal calcium concentration is above the residual value shown above.

$$\frac{d[Ca^{2+}]_i}{dt} = -0.000015I_{Ca} - \frac{1}{\tau}([Ca^{2+}]_i - 0.0001) \qquad (5)$$

Table 2 shows the gating variables below for the four channels that are voltage-dependent. All of the variables act in a similar manner to the following first order equation:

$$\frac{dx}{dt} = -(\alpha_x + \beta_x)x + \alpha_x \qquad (6)$$

This equation shows how the channel can be inactivated/activated. The Sodium-gated channel is dependent on the following variables: $\alpha_m, \beta_m, \alpha_h, \beta_h$. While the calcium gated channel is gated by α_c, β_c. The potassium channel is gated by: α_n, β_n. Lastly, the A channel is gated by these variables: $\alpha_A, \beta_A, \alpha_{hA}, \beta_{hA}$.

$\alpha_m = -\frac{0.6(V+30)}{\exp(-0.1(V+30))-1}$	$\beta_m = 20\exp\left(\frac{V+55}{18}\right)$
$\alpha_h = 0.4\exp\left(-\frac{V+50}{20}\right)$	$\beta_h = \frac{6}{\exp(-0.1(V+20))+1}$
$\alpha_n = -\frac{0.02(V+40)}{\exp(-0.1(V+40))-1}$	$\beta_n = 0.4\exp\left(-\frac{V+50}{80}\right)$
$\alpha_c = -\frac{0.3(V+13)}{\exp(-0.1(V+13))-1}$	$\beta_c = 10\exp\left(-\frac{V+38}{18}\right)$
$\alpha_A = -\frac{0.006(V+90)}{\exp(-0.1(V+90))-1}$	$\beta_A = 0.1\exp\left(-\frac{V+30}{10}\right)$
$\alpha_{h_A} = 0.04\exp\left(-\frac{V+70}{20}\right)$	$\beta_{h_A} = \frac{0.6}{\exp(-0.1(V+40))+1}$

Table 2. Rate Constants for Na^+, Ca^{2+}, K^+, A Channels [5]

Some other parameters that we looked into are changing the calcium and sodium conductances in relation to the drug betaxolol.

4. RESULTS

4.1 Calcium Time Constant Tuning

To see how the time constant (τ) can change the calcium concentration we observed the membrane voltage and concentration graphs over time for a high and low time constant. When τ is equal to 5, we observed that it is similar to the biological data of a spike train of a retinal ganglion cell when stimulated by the 10pA as shown in Figure 3(a) [5]. In Figure 4, the τ is set to 500, and we can see that this is more similar to the RGC stimulated by 40pA. Comparing the two graphs below you can see that when the time constant is lowered there is an exponential increase in the calcium concentration.

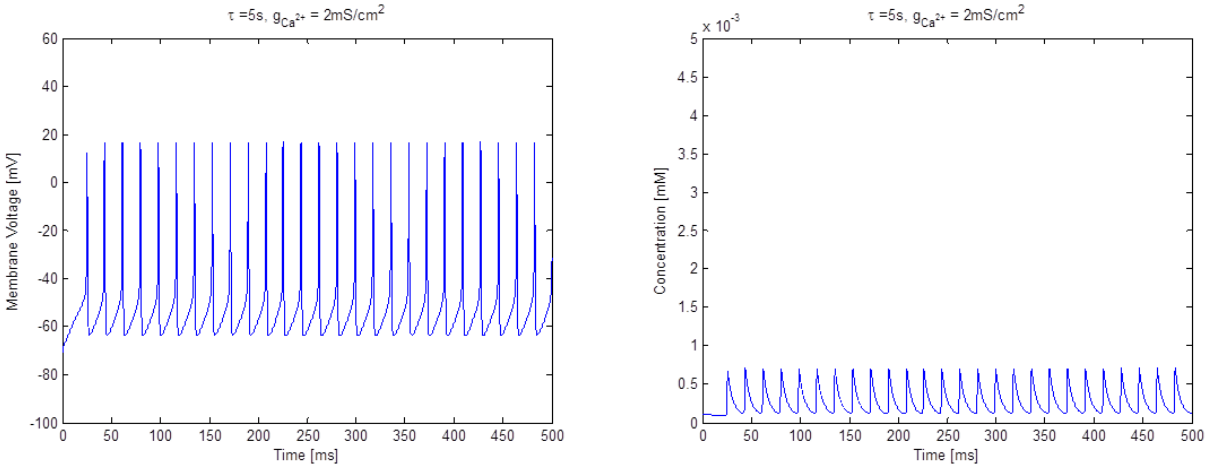

Figure 3(a) Membrane Voltage with τ= 5s. (b) Concentration with τ= 5s

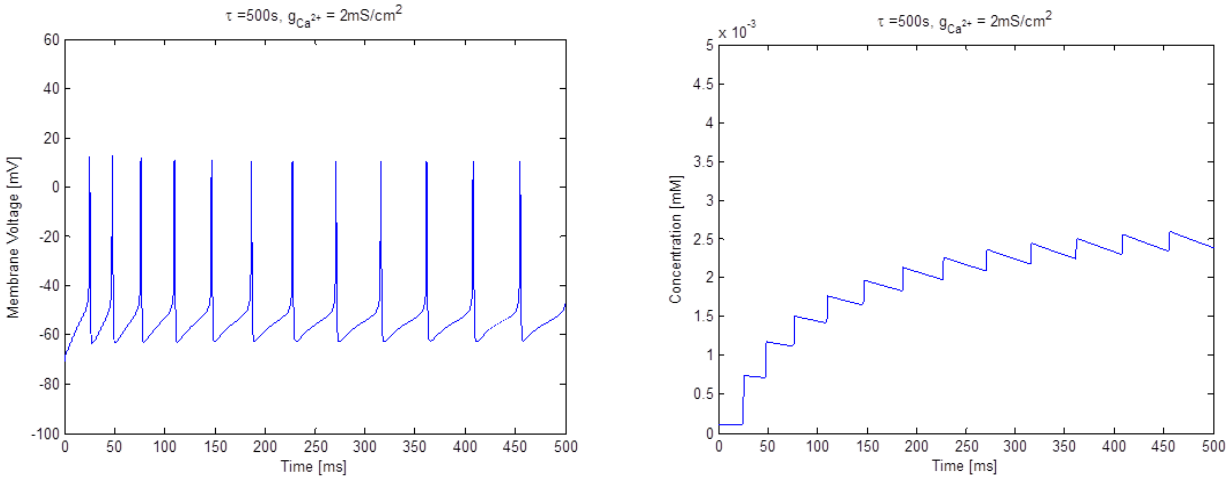

Figure 4(a) Membrane Voltage with τ= 500s. (b) Concentration with τ= 500s

4.2 Betaxolol Tuning: Varying Sodium and Calcium Conductance

Application of 50mM betaxolol to isolated RGC reduced the voltage-gated sodium and calcium currents by approximately one third of their peak amplitudes [7]. When the sodium conductance is decreased by one-third of its original value and the calcium conductance is held constant, the spiking frequency drops to about 44Hz (Fig. 5) from 49Hz (Fig. 6). If we model peak amplitude

decrease of sodium and calcium currents by decreasing the respective conductance by one-third, the drug does not elicit any beneficial effect in regards to lowering spiking frequency (Fig. 7). A significant drop in peak membrane voltage is observed when sodium conductance is dropped; a possible explanation is that the lowered sodium conductance prevents the membrane potential from being driven towards the reversal potential of sodium ions during depolarization.

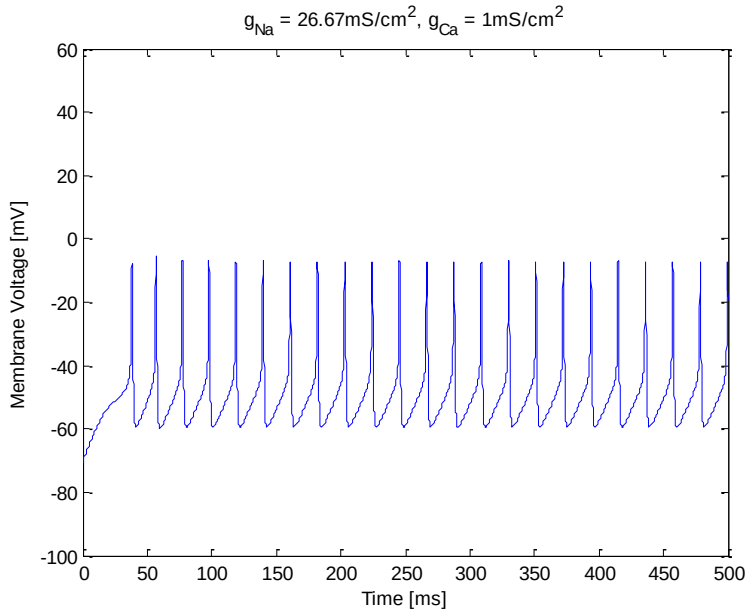

Figure 5. Membrane Voltage with g_{Na} = 26.67mS/cm^2, g_{Ca} = 1 mS/cm^2

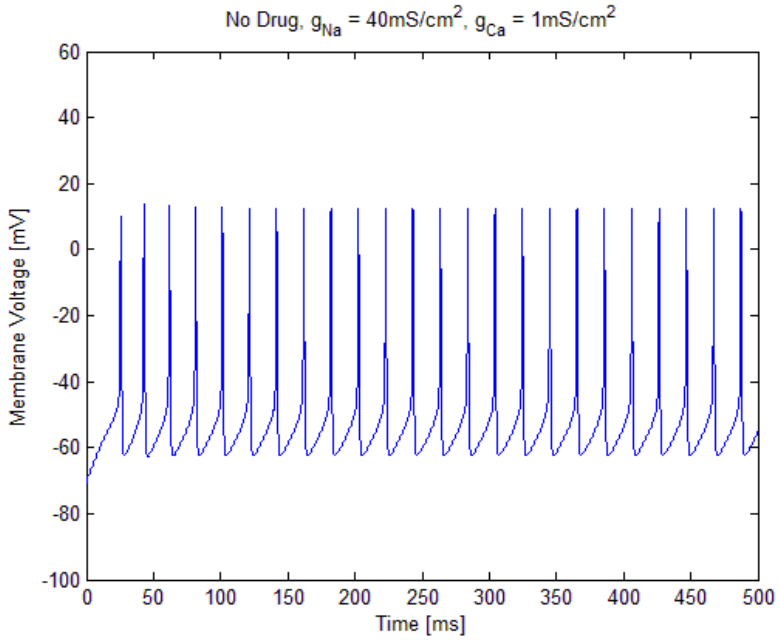

Figure 6. Membrane Voltage with g_{Na} = 40mS/cm^2, g_{Ca} = 1 mS/cm^2

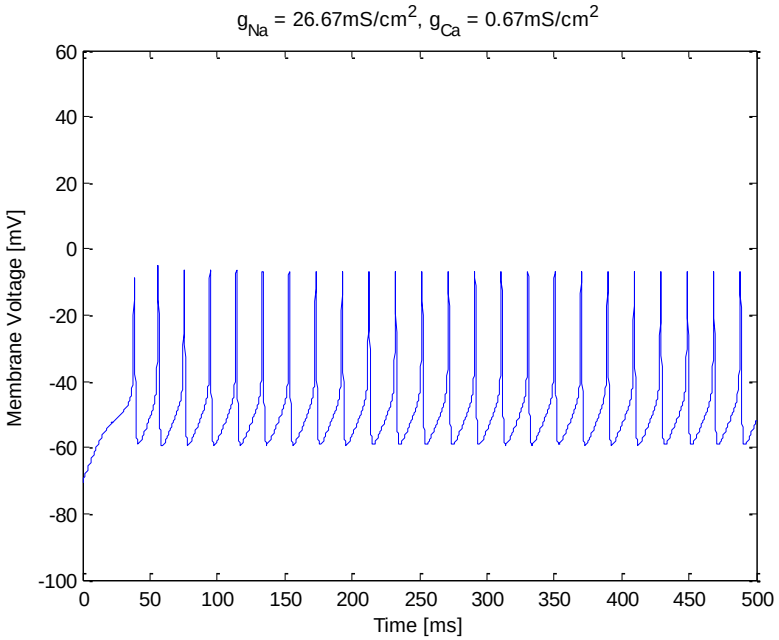

Figure 7. Membrane Voltage with g_{Na} = 26.67 mS/cm^2, g_{Ca} = 0.67 mS/cm^2

5. DISCUSSION

Understanding the gating dynamics of a single retinal ganglion cell can foster research in the treatment of retinopathy. Retinopathy is damage to the phototransduction through usually hypertension or diabetic retinopathy. Glaucoma, an increase in a patient's intraocular pressure, is treated using betaxolol. Betaxolol is a selective beta-1 adrenergic receptor blocker that selectively targets sodium ion channels. Most importantly, betaxolol has no effect on horizontal cells nor in an electroretinogram [7]. Therefore, betaxolol only affects the retinal ganglion cells which is how we composed our model. By blocking the channels, betaxolol lowers the firing frequency of the RGC. However, betaxolol inadvertently also targets calcium ion channels [7]. If the effect on calcium conductance outweighs the reduction in sodium conductance, then drugs like betaxolol may not be effective treatments for patients with retinopathy.

When the sodium conductance of the cell is decreased the firing frequency also drops. Normally, sodium conductance is near 40mS/cm^2 [7]. When sodium conductance is dropped by 25% the firing frequency settles at 45Hz. Conversely, when calcium conductance is lowered the RGC spiking frequency increases.

6. CONCLUSION

To gain an understanding of how varying conductances alter ganglion firing behavior, this project analyzes the effects of both calcium and sodium channel blockers, specifically betaxolol on a single RGC's firing behavior. Due to the limitations of the model, such as the lack of including variations from cells upstream and that channel blockers will likely have effects on other cells as well as RGCs, this model only works well for the biological data stimulated by 20pA and 1uA/cm^2. For future models, to have a more realistic model cells that are upstream in the phototransduction pathway of the RGCs can be incorporated. Specifically, the circuits designed for the bipolar and amacrine cells could be analyzed and then be connected to the existing model through the stimulation current.

7. References

[1] Rodieck, Robert W. "Quantitative analysis of cat retinal ganglion cell response to visual stimuli." *Vision research* 5.12 (1965): 583-601.

[2] Famiglietti, E. V., and Helga Kolb. "Structural basis for ON-and OFF-center responses in retinal ganglion cells." *Science* 194.4261 (1976): 193-195.

[3] Hubel, David H., and Torsten N. Wiesel. "Receptive fields of single neurones in the cat's striate cortex." *The Journal of physiology* 148.3 (1959): 574.

[4] Granit, Ragnar, and P. O. Therman. "Excitation and inhibition in the retina and in the optic nerve." *The Journal of physiology* 83.3 (1935): 359-381.

[5] Fohlmeister, J. F., P. A. Coleman, and R. F. Miller. "Modeling the repetitive firing of retinal ganglion cells." *Brain research* 510.2 (1990): 343-345.

[6] Silver, Ian A., and Maria Erecinska. "Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons." *Journal of neurophysiology* 79.4 (1998): 1733-1745.

[7] Gross, Ronald L., et al. "Effects of betaxolol on light responses and membrane conductance in retinal ganglion cells." *Investigative ophthalmology & visual science* 41.3 (2000): 722-728.

[8] Maturana, Matias I. et al. "The Effect of Morphology Upon Electrophysiological Responses of Retinal Ganglion Cells: Simulation Results." *Journal of Computational Neuroscience* 36.2 (2014): 157–175. *PMC.* Web. 18 Dec. 2014.

[9] Hodgkin A.L. and Huxley, A.F. "A quantitative description of membrane current and its application to conduction and excitation in nerve." *J. Physiol. (Lond.)* 117(1952):500-544