Computational Optogenetics: Reducing Hyperexcitability of neurons through the use of Channelrhodopsin 2

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Abstract

In the scope of this research project we implement a computational model for the dynamical characteristics of channelrhodopsin 2, which was developed by the Cardiac Optogenetics and Optical Imaging Lab at Stony Brook University. We aim to determine the feasibility of using ChR2 to control and decrease the hyper excitation of neural networks.

1 Background

Channelrhodopsin 2 (ChR2) is a light sensitive protein that can act as a light gated ion channel, which can provide the ability to modulate neural activity. ChR2 provides a depolarizing ionic current after illumination by light at a wavelength of 470 nm corresponding to blue light. The photocycle of ChR2 has been extensively studied by numerous research groups and lots of empirical data has been collected pertaining to its characteristics e.g. current flow as a result of light stimulation. This has allowed for the development of an empirically based computational model of ChR2 developed by the computational Optogentics group at the Stony Brook University.

The applications of computational optogenetic models is far reaching not only in the field of neuroscience, but also in the field of cardiology as shown by Williams et al. [1]. Having these computational models for opsins such as ChR2 can allow neuroscientists to test and validate in simulation, the effects of adding ChR2 light gated ion channels into neural networks. Since these channels are light activated, precise stimulation and control can be achieved to stimulate specific sections of the brain using fiber optic and laser diode technology. In addition it has proven effective in aiding in the functional mapping of neurons and determining their connectivity [1].

Computational neuroscience has allowed for a more in depth understanding and modeling, of the dynamics of individual neurons and furthermore large neural networks. With the existing channelrhodopsin model, we can begin to evaluate the level of control optogenetics provides. For example, epilepsy is thought to be a disease caused by recurrent excitation created by a change in the topography of a neural network [2]. Controlling networks with recurrent excitation may lead to seizure prevention or cessation. Optogenetic transfection in these networks may allow for production of an optical neural pacemaker. We will evaluate the level of control channelrhodopsin provides in both a four neuron model and a larger model.

2 Methods

2.1 Optogenetics model

Studies analyzing the ChR2 and it's photocycle have have lead to the proposal of two models for it's photocycle. The two primary models in current literature include a three state model and an updated four state model (fig. 1). Williams et al. adapted the four state model proposed by Nikolic et al. and developed their computational model based off of it. Using the four state Markov model proposed in [3], Williams et al. fitted empirical ChR2 dynamics data obtained over a wide range of irradiances and voltage values to the model and obtained values for the various parameters governing the four state model. These are the values which we have employed in our recreation of the model.

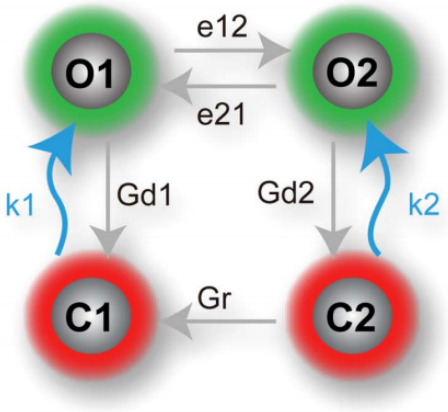

Figure 1: ChR2 Photocycle Markov Model [2]

The four state model is described by two open states and 2 closed states all of which have rate constants associated with them. The following first order, ordinary differential equations, derived and developed in [1], govern the dynamic characteristics of ChR2. Where C1,C2,O1 and O2 represent the probabilities of the ChR2 molecule being in each of the respective states and where the sum C1 + C2 + O1 + O2 = 1.

$$dC1/dt = Gr\,C2 + Gd1\,O2 - k1C1$$
$$dC2/dt = Gd2\,O2 - (k2 + Gr)C2$$
$$dO1/dt = k1\,C1 - (Gd1 + e12)\,O1 + e21\,O2$$
$$dO2/dt = k2\,C2 - (Gd2 + e21)O2 + e12\,O1$$

The final voltage dynamics of the neuron transfected with the ChR2 is given by the following equation, which is the Hodgkin and Huxley model, to express the neuron voltage with the addition of the ChR2 current value.

$$dV/dt = (1/C_m)(I_{Na}(V) - I_K(V) - I_L(V) - I_{ChR2}(O1, O2, V) - I_{excitatory}(V)$$

Where $I_{ChR2}(O1, O2, V)$ is given by

$$I_{ChR2}(O1, O2, V) = g_{ChR2}\,G(V)(O1 + \gamma O2)(V - E_m)$$

Which is a function of the probability of the ChR2 molecule being in the open 1 (O1) state, open 2 (O2) state, the

conductance of the ChR2 channel, its membrane voltage a voltage dependent scaling factor G and γ the ratio of the conductances of the O1 and O2 states. The current response to our updated version can be found in section 3.1. Including these coupled ODE's into our ODE solver in MATLAB. Along with functions provided by Williams et al. we were able to recreate the current response of ChR2 to specific illumination cases. One caveat, to implementing the ChR2 current dynamics into the Hodgkins-Huxley model we used was the need to increase the conductance in the ChR2 current equation in order to induce spiking at 5 mW of light illumination.

2.2 Four neuron model

Using MATLAB, we constructed a four neuron Hodgkin-Huxley model to explore optogenetics in a small network with recurrent excitation. We will explore the behavior of the Figure 1 model and an equivalent model with a damaged neuron A. The feedback loop and the damage to A represents pathologies that may arise through head trauma, The model was constructed to exemplify a specific situation where we suspect channelrhodopsin should be effective. Channelrhodopsin 2 produces an excitatory effect upon optical stimulation. For this reason, to reduce activity in the network, the inhibitory neuron B is powered by a light source. The reduction in the spiking frequency of C will be observed. The simulation was executed for two seconds. The rest of the simulation parameters are listed in Tables 1 and 2.

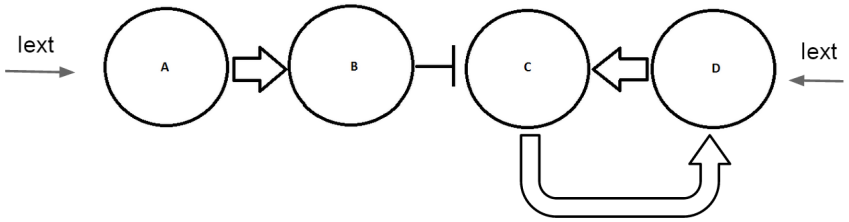

Figure 2: Undamaged Four Neuron Model with Positive Feedback

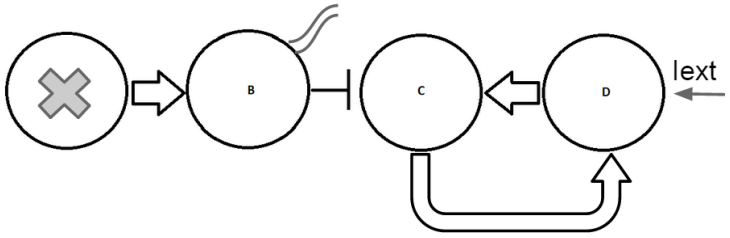

Figure 3: Damaged Four Neuron Model with Positive Feedback

2.3 Regular and small world network model

To more realistically model a situation in which the channelrhodopsin 2 is used, we constructed two larger networks: a regular network and a small world network. Both networks were generated using the freely available "Biological Neural Networks Toolbox" for MATLAB. We chose to use these networks because previous research has shown realistic epileptiform activity arising from small world networks of 3,000 neurons, thus controlling this type of network is likely to extend to more realistic models.[3] For our purposes, the regular network consists of a ring of 32 Hodgkin-Huxley neurons which are only connected to the nearest neighbors. The connections made with Neurons 7 and 27 are all inhibitory. The small world network consists of the same neurons as the regular network, but new long-range synaptic connections are randomly generated. Long range connections had a longer synaptic delay and lower

conductances. Connections were randomly generated such that each neuron has an integer K connections. The random connections were generated through MATLAB's uniform random distribution function. Simulations were driven by a single pulse of current at neuron one and run for 100ms. Optical stimulation was only applied to the small world network. Trials with stimulation applied had the optical wavelength at 470nm and a constant 1 mW/mm^2 irradiance.

Parameter	Parameter Value
$Iext_A$	9 μA
$Iext_B$	14 μA
Excitatory α	$2.4mM^{-1}ms^{-1}$
Excitatory β	$.56ms^{-1}$
Excitatory Tmax	1.5mM
Inhibitory α	$5mM^{-1}ms^{-1}$
Inhibitory β	$.18ms^{-1}$
Inhibitory Tmax	1.0mM

Table 1

Parameters	Parameter Value
Neuron Capacitance	$1\ \frac{\mu F}{cm^2}$
E_K	-82mV
E_Na	45mV
E_L	-59.387mV
E	-38mV
Optical Stimulation Delay	100ms
Optical Stimulation Duration	300ms
Wavelength	470nm

Table 2

Tables 1 and 2: Model Parameters

3 Results and Discussion

3.1 Optogenetics model

The figure below shows the current response of the ChR2 versus time. Again, using the empirically derived constants from [1] we were able to obtain a realistic current response of the ChR2 that we could include into the Hodgkin - Huxley model in order to gate the dynamics of our overall neural network with simulated light pulses. After our modifications, the results still behaved as expected, with the characteristic high pulse of initial current which eventually reaches a steady state. When the optical stimulation is turned off at 400 ms, the current rapidly returns to zero. Subsequent pulses do not exhibit the high initial pulse of current, which coincides with the well-known memory effect of channelrhodopsin 2.

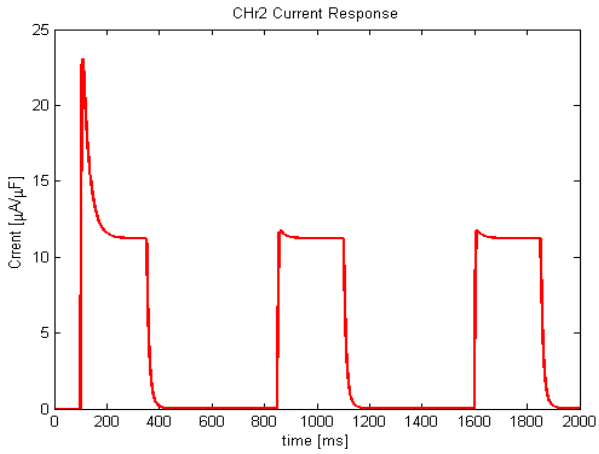

Figure 4:ChR2 current response after stimulation with 2.5 mW, 470 nm light source lasting 300ms per pulse

3.2 Four neuron model

As shown in figure 5, the normally functioning network had Neuron C spiking at a rate between 13-20Hz, even with the recurrent excitation. However, when the inhibitory neuron B was no longer powered by Neuron A, as shown in figure 6, the spike rate of C increased dramatically to 60-70Hz. The recurrent excitation does not cause a runaway gain of spike rate because the neurons quickly reach the equilibrium spiking rate that is characteristic of Hodgkin-Huxley neurons.

When we introduced optical stimulation at 100ms, 900ms, and 1600ms for a duration of 300ms, we observe a beneficial 78Hz spike rate in Neuron B. This reduces the spiking rate of C to zero after a 100ms delay. Our result for this model demonstrates the ability for channelrhodopsin 2 to significantly reduce the spiking rate in the presence of positive feedback, given an inhibitory neuron is not initially spiking. This method allows for rapid control of the network as well, a change in the optical stimulation is seen in the recorded neuron in less than 100ms. The characteristic spike in current due to the channel response does not prove to be an obstacle in our level of control, as it produce any visible effects in the network output. However, in attempting finer control of the network this characteristic response may prove to be limiting. It should also be noted that in this model the channelrhodopsin is able to express the greatest inhibitory effect because the inhibitory neuron starts from a resting state.

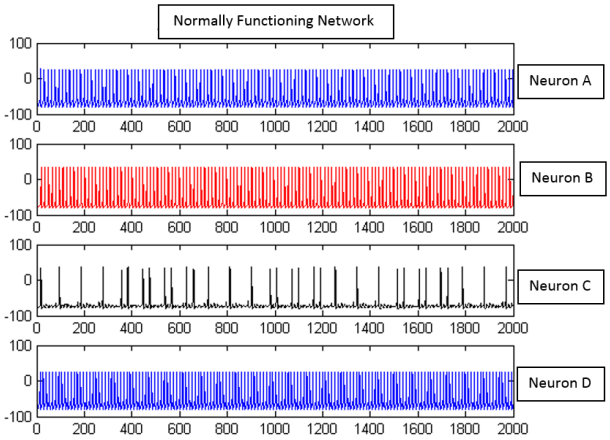

Figure 5

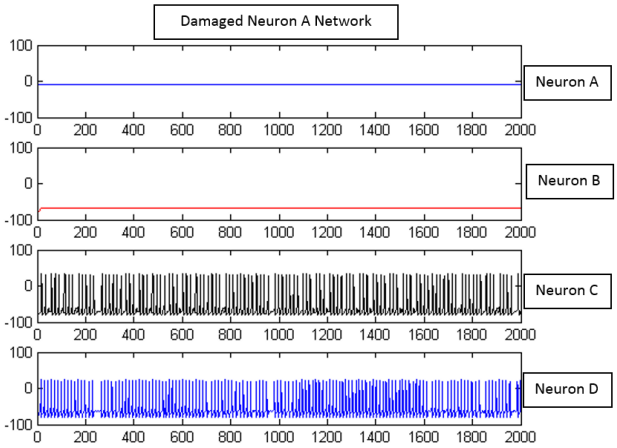

Figure 6

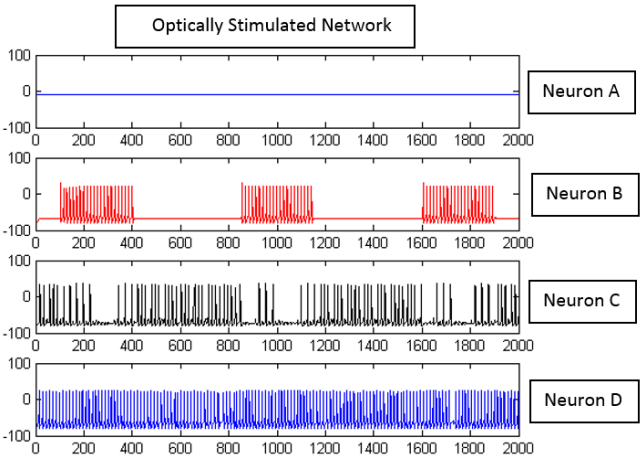

Figure 7: Damaged Model with Optical Stimulation

3.3 Regular and small world network model

The regular network behaved as expected, with the pulse of current only causing a single spike in each neuron until the spike train reached the inhibitory neurons. No further activity occured in the network. The more interesting result, shown in figure 9, shows the small world network where each neuron has three long range connections. The network is again powered by just a single pulse, but the network now shows continuous activity. This is because the long range connections have enough of a delay for the spike to reach the distant neuron once it has finished its refractory period.

The inhibitory neurons in the network do not spike often enough to inhibit the network as a whole. Examining figure 9 shows that the neurons with connections to the inhibitory neurons have a lower spiking rate, so there is an inhibitory effect, but it is very small. When we try to increase the inhibitory effect by introducing the opsin dynamics into the network, figure 10 shows the spike rate of the inhibitory neurons are increased, and the neurons directly connected to the inhibitory neurons have a decreased spiking rate. This effect does not propagate throughout the whole network. This is in stark contrast to our previous model where the opsin dynamics allowed us to dramatically reduces the output of the network. In this case, the controlling power of the opsin channel is severely diminished because the inhibitory neurons are already included in the recurrent excitation. Even without the opsin dynamics the inhibitory neurons spike at a rapid rate, and since Hodgkin-Huxley neurons reach an equilibrium spiking rate with increased current, the current through the opsin channels simply does not produce a large effect on the overall network.

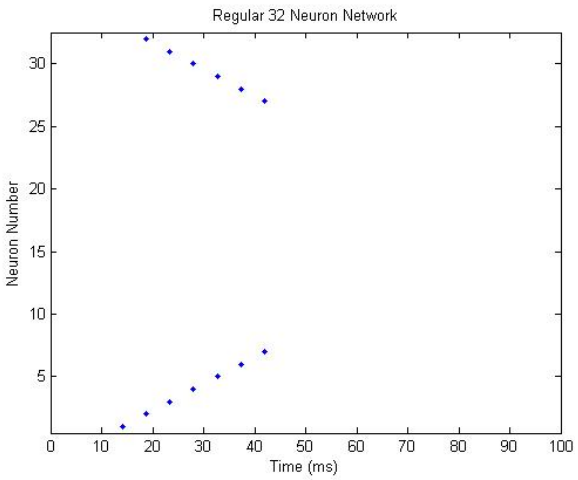

Figure 8: Regular network powered by a single pulse of current

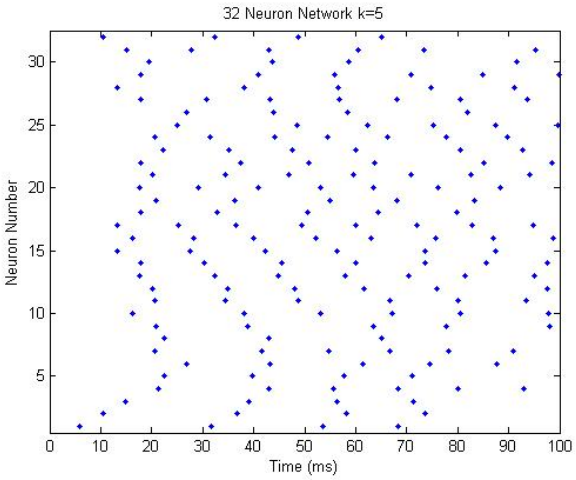

Figure 9: Small world network with no optical stimulation

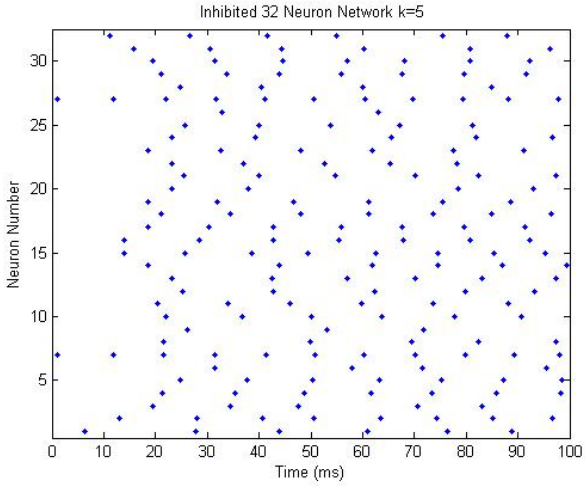

Figure 10: Small world network with optical stimulation

4 Conclusion

The efficacy of channelrhodopsin 2 at inhibiting neural networks largely depends of the topography of the network and the properties of the inhibitory neurons. Our models have shown that ChR2 is most effective when the transfected inhibitory neuron initially has a low spiking rate. This means for optimal results, inhibitory neuron must be outside of the region with high activity, yet still control the region. This condition is a major limitation of using an excitatory opsin to increase inhibition, thus ChR2 is not an ideal candidate for use with an optical pacemaker. Further neural pacemaker research should explore the utility of the inhibitory opsin, halorhodopsin, for more promising results [4]. Using halorhodopsin would allow any excitatory neuron in the region to be transfected, which will both reduce the specificity needed in the transfection process, and may also allow greater inhibition in the network. Research should begin with studying the photocycle of halorhodopsin in order to proceed with the formulation of a halorhodopsin computational model.

5 References

[1] Williams, John C., Jianjin Xu, Zhongju Lu, Aleksandra Klimas, Xuxin Chen, Christina M. Ambrosi, Ira S. Cohen, and Emilia Entcheva. "Computational Optogenetics: Empirically-Derived Voltage- and Light-Sensitive Channelrhodopsin-2 Model." Ed. Mark S. Alber. *PLoS Computational Biology* 9.9 (2013): E1003220. Web.

[2] Netoff, T. I. "Epilepsy in Small-World Networks." *Journal of Neuroscience* 24.37 (2004): 8075-083. Web.

[3] Nikolic, Konstantin, Nir Grossman, Matthew S. Grubb, Juan Burrone, Chris Toumazou, and Patrick Degenaar. "Photocycles of Channelrhodopsin-2." *Photochemistry and Photobiology* 85.1 (2009): 400-11. Web.

[4] Yizhar, Ofer, Lief E. Fenno, Thomas J. Davidson, Murtaza Mogri, and Karl Deisseroth. "Optogenetics in Neural Systems." *Neuron* 71.1 (2011): 9-34. Web.