The Propagating Action Potential in the Squid Giant Axon

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***Abstract*—Signals are propagated within biological systems through action potentials, which occur when the membrane potential of an excitable cell is depolarized. This initial depolarization begins a chain reaction, known as a spike train, in which the initial signal causes action potentials to occur down the length of an axon. These impulses are produced and regulated by voltage gated ion channels, usually sodium and potassium channels, embedded within the plasma membrane of an excitable cell. The Hodgkin-Huxley model effectively describes these mechanisms through a series of nonlinear differential equations. Here, we utilize this model to determine the velocity of an action potential propagated along the length of a giant squid axon with a length of 10cm and spatial steps of 0.05cm.**

I. Introduction

The Hodgkin-Huxley Model is composed of a series of nonlinear differential equations that describe how action potentials are commenced and propagated through excitable cells. Within this model, each biological structure within a neuron is expressed as a mathematical variable. Specific to a giant squid neuron, the elements are described as follows: the lipid bilayer as capacitance (Cm), voltage gated ion channels by conductances (g), each electrochemical gradient as a voltage source (En), and the membrane potential as Vm. Together, these fundamental components can be used to describe the total current through a membrane as I=Cm(dVm/dt)+gk(Vm-Ek)+gNa(Vm-ENa)+gl(Vm-Ek), where gl represents the leak channels.

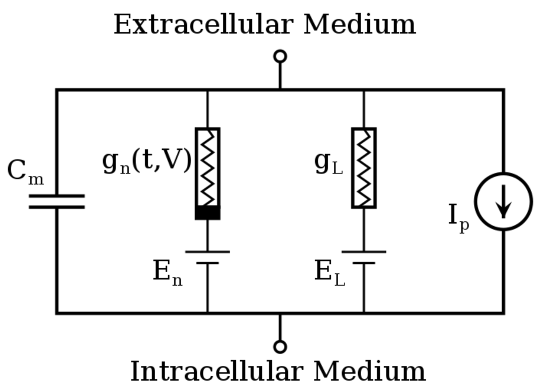

Fig. 1: Hodgkin Huxley Cell Membrane Circuit Model. Obtained from [1]

After conducting a series of voltage clamp experiments, Hodgkin and Huxley further refined their initial model to include the following four differential equations:

$$I = C_m \frac{\mathrm{d}V_m}{\mathrm{d}t} + \bar{g}_K n^4 (V_m - V_K) + \bar{g}_{Na} m^3 h (V_m - V_{Na}) + \bar{g}_l (V_m - V_l),$$
$$\frac{dn}{dt} = \alpha_n(V_m)(1-n) - \beta_n(V_m)n$$
$$\frac{dm}{dt} = \alpha_m(V_m)(1-m) - \beta_m(V_m)m$$
$$\frac{dh}{dt} = \alpha_h(V_m)(1-h) - \beta_h(V_m)h$$

Fig. 2: Hodgkin Huxley State Equations. Obtained from [2].

Within these expressions, the conductance g is now expressed as the maximal value of conductance multiplied by the probability of that specific gate being open. In this new model, there are four n gates that represent four sodium channels with a probability of n raise to the fourth power, and three m gates along with one h gate to denote the potassium channels which equates to a probability of m raised to the third power times h. Because this advanced model incorporates probability, it became necessary to account for the opening and closing rates of each type of gate, with alpha being used to denote the opening rate and beta for the closing rate, both having units of msec-1. These rates are derived from the following expressions, and they change with a new membrane voltage per each time step:

$$\alpha_n(V_m) = \frac{0.01(10-V_m)}{\exp\left(\frac{10-V_m}{10}\right)-1} \qquad \alpha_m(V_m) = \frac{0.1(25-V_m)}{\exp\left(\frac{25-V_m}{10}\right)-1} \qquad \alpha_h(V_m) = 0.07\exp\left(\frac{-V_m}{20}\right)$$
$$\beta_n(V_m) = 0.125\exp\left(\frac{-V_m}{80}\right) \qquad \beta_m(V_m) = 4\exp\left(\frac{-V_m}{18}\right) \qquad \beta_h(V_m) = \frac{1}{\exp\left(\frac{30-V_m}{10}\right)+1}$$

Fig. 3: Hodgkin Huxley Rate Equations. Obtained from source [2]

II. Methods

A. Mathematical Process

A MATLAB simulation was designed to mimic an action potential propagating down the length of a giant squid axon. In order to determine the velocity of a propagated action potential, we first utilized the equation

$$\frac{\partial V_m}{\partial t} = \frac{1}{2\pi a (r_i + r_o) C_m} \frac{\partial^2 V_m}{\partial z^2} - \frac{1}{C_m}\left[J_{ion} - J_m\right]$$

Fig. 4: Change in Voltage due to Currents and Diffusion.

which is a rearranged form of the Cable Equation known as the Reaction-Diffusion Equation. Within this equation, Jion originates from the Hodgkin Huxley Model, a represents the radius of the axon (0.025cm in our experiment), and ri+ro describe resistance per unit length of the intra and extracellular fluids. Like in the Hodgkin Huxley Model, this equation is solved using the finite difference method which accounts for changes in both time (t) and space (z). Their respective derivatives, along with their placement into the Reaction-Diffusion Equation, are listed below:

$$\frac{\partial V_m}{\partial t} = \frac{\partial V_j^i}{\partial t} \approx \frac{V_j^{i+1} - V_j^i}{\Delta t}$$
$$\frac{\partial^2 V_m}{\partial z^2} = \frac{\partial^2 V_j^i}{\partial z^2} \approx \frac{V_{j+1}^i - 2V_j^i + V_{j-1}^i}{(\Delta z)^2}$$

Fig. 5: Change in Voltage Derivation

When applied, this equation can be solved to show the new value of Vm at location j per time step i+1 by the following expression:

$$\frac{\partial V_j^i}{\partial t} \approx \frac{V_j^{i+1} - V_j^i}{\Delta t} = D\left[\frac{V_{j+1}^i - 2V_j^i + V_{j-1}^i}{(\Delta z)^2}\right] + \frac{1}{C_m}\left[J_m - J_{ion}\right]$$

Fig. 6: Final Change in Voltage Equation

Per each iteration of this formula, we computed new Jion value at each time step and saved the state variables (Vm, n, m, and h) for each segment along the axon. After registering these values, we were then able to simulate a 10cm long giant squid axon. Our conditions applied a stimulus current of 50 microA/cm2 for 4 milliseconds to axon segments 2, 3, and 4, a spatial step deltaz of 0.05cm, and a criterion as follows for each time and spatial step:

$$\frac{\Delta t}{2\pi a\left(r_i + r_o\right) C_m (\Delta z)^2} \leq \frac{1}{2}$$

Fig. 7: Timestep Constraint Equation

In our experiment, we instituted no flux boundary conditions, which state that no current flows out of either end of the axon. Mathematically, this means that Vm(1)=Vm(2) and Vm(L)=Vm(L-deltaz). After simulating and plotting these Vm values across the length of the axon, we recorded the activation time, which is when Vm is greater than -30mV at the locations z=L/2 and 3L/4. Using these values, we were able to use the following equation to determine the propagation velocity:

$$\theta = \frac{dz}{dt} \approx \frac{\Delta z}{\Delta t} = \frac{3L/4 - L/2}{(\text{activation time at } 3L/4) - (\text{activation time at } L/2)}$$

Fig. 8: Equation for Propagation Velocity

B. *Matlab Implementation*

To implement this mathematical process in matlab, we first defined a set of parameters:

Conductances, Nernst Potentials, and Capacitances:

- gk=36.0mS
- gNa=120mS
- gL=0.3mS
- Ek=-72.1mV
- ENa=52.4mV
- EL=-49.187mV
- Cm=1.00uF
- Ro=0Kohm/cm
- Ri=5Kohm/cm
- a=0.025cm

Initial State Variable Values:

- n=0.31768/msec
- m=0.05293/msec
- h=0.59612/msec
- Vrest= -60mV

After establishing these constants, we created arrays to store each state variable (and some associated variables) at each time step. A time loop iterated every 0.02ms was then initiated. During this time loop, a space loop was embedded which would calculate the change in each state variable for each segment of the axon. After this the changes in state variables were added to the previous values (again for each segment of the axon) in arrays called Vmnew, nnew, mnew, and hnew. After the conclusion of the space loop, a step forward in time was taken. For each step forward in time, the "new" state variable arrays were cycled the respective Vm, n, m, and h arrays to be stored as the new "previous values". Additionally, the Vm arrays for each step in time were stored in their own matrix for plotting. This matrix was then plotted against both space for each time step, with a "drawnow" command forcing the graph to show the Vm values at each time step. A separate plot graphed Vm versus time at two sections on the axon. These two plots were then used to compute the difference in space over difference in the difference in time for each of the action potentials on the two axon sections, which equates to the velocity of the action potential. After deriving this value, we ran our script with a variety of ri values ranging between 5 and 30 Kohm/cm in order to determine the effect that it would have on the velocity of an action potential. Our script for this simulation is posted within the appendices.

C. *Computer Specifications*

- Matlab Version: R2017b
- Processor: Intel Core i5-5200 CPU 2.20GHz
- RAM: 8.00GB
- Operating System: Windows 8

III. Results

The following graph depicts an action potential traveling down the length of a 10cm giant squid axon. Its resting potential is -60mV, and when depolarized its membrane potential rises to +40mV. As the potential progresses along the axon, each sequential segment is depolarized by the preceding segment.

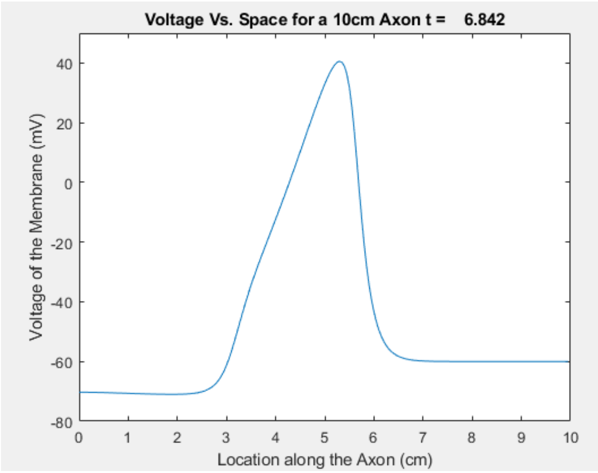

Fig. 9: Action Potential propagation

Below are our results for the dV/dtmax, APD90 at the two segments, and propagation velocity for each value of ri.

TABLE I

ri	dv/dtmax	APD90 L/2	APD90 3L/4	Velocity
5	210.31	0.894	0.897	2.3481
10	210.31	0.912	0.915	1.6611
15	210.31	0.8932	0.8932	1.3554
20	210.31	0.8816	0.8816	1.1708
25	210.31	0.9028	0.8991	1.0447
30	210.31	0.8932	0.8932	0.9527
Kohm/cm	mV/msec	msec	msec	cm/msec

Our various action potential graphs for voltage vs space and voltage vs. time at different values of ri are as follows:

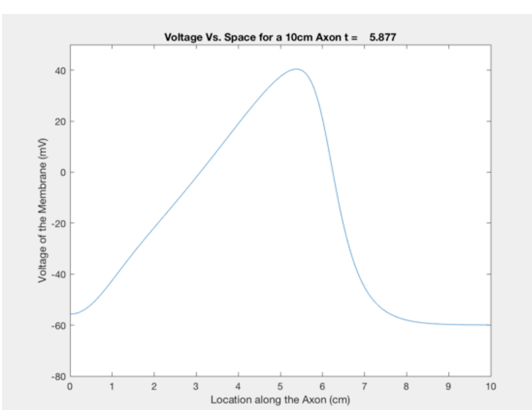

Fig. 10: Action Potential propagation when Ri=5Kohm/cm

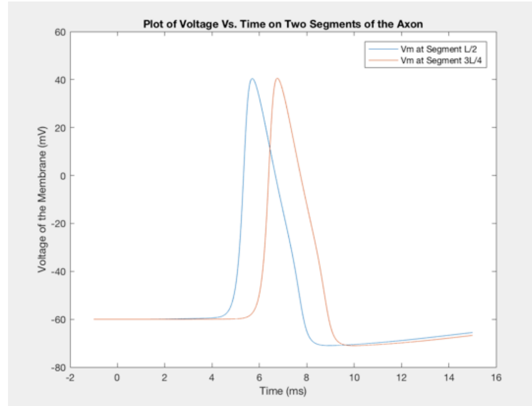

Fig. 11: Voltage vs Time when Ri=5Kohm/cm

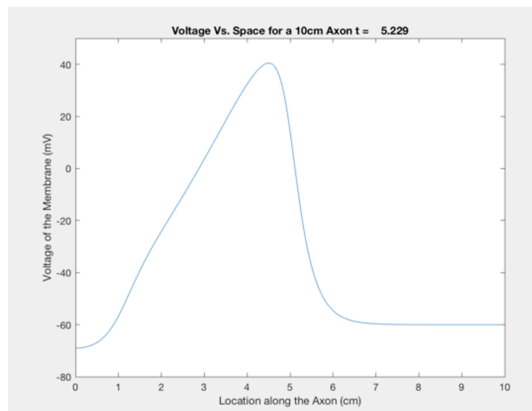

Fig. 12: Action Potential propagation when Ri=10Kohm/cm

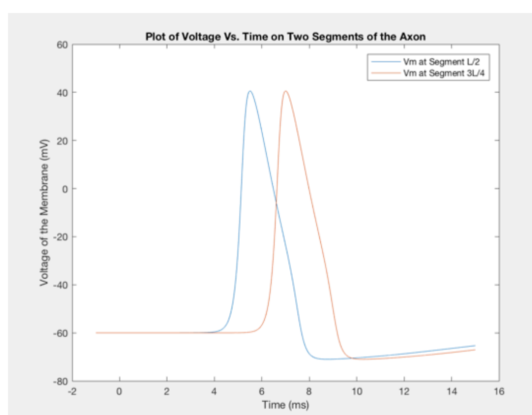

Fig. 13: Voltage vs Time when Ri=10Kohm/cm

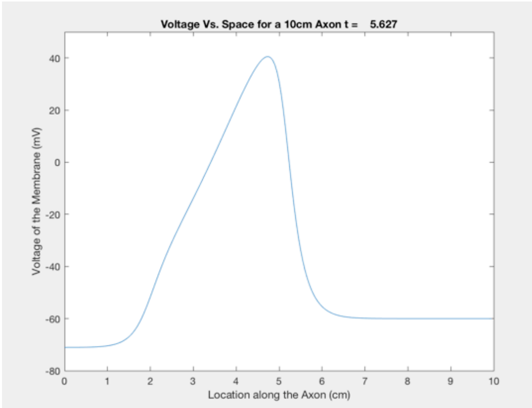

Fig. 14: Action Potential propagation when Ri=15Kohm/cm

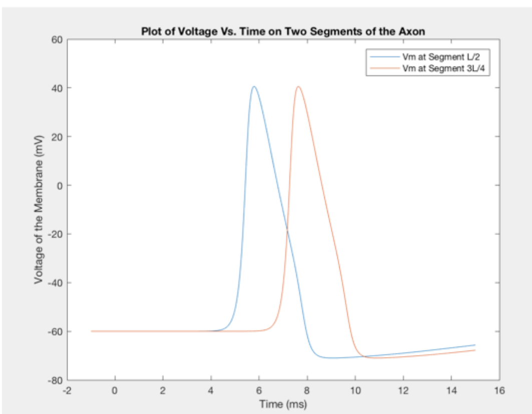

Fig. 15: Voltage vs Time when Ri=15Kohm/cm

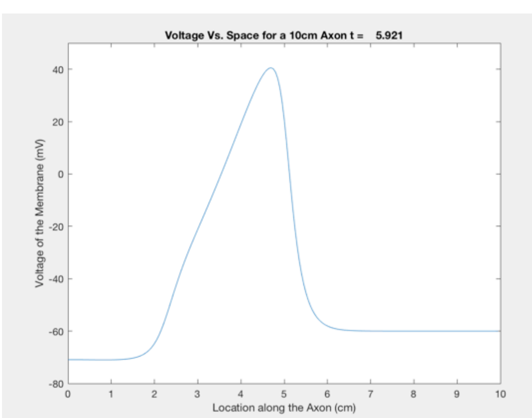

Fig. 16: Action Potential propagation when Ri=20Kohm/cm

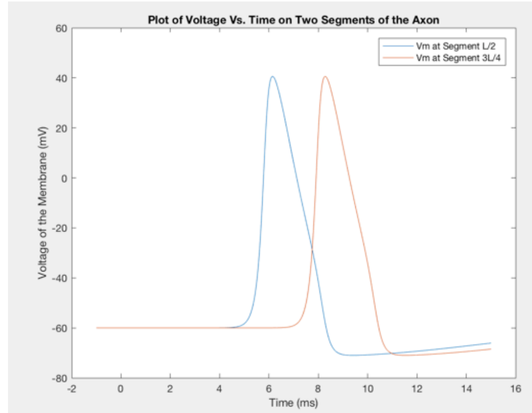

Fig. 17: Voltage vs Time when Ri=20Kohm/cm

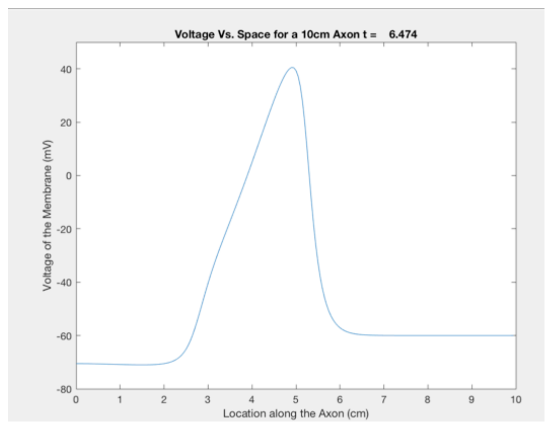

Fig. 18: Action Potential propagation when Ri=25Kohm/cm

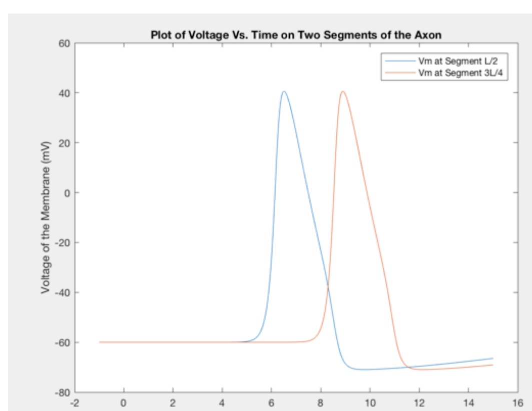

Fig. 19: Voltage vs Time when Ri=25Kohm/cm

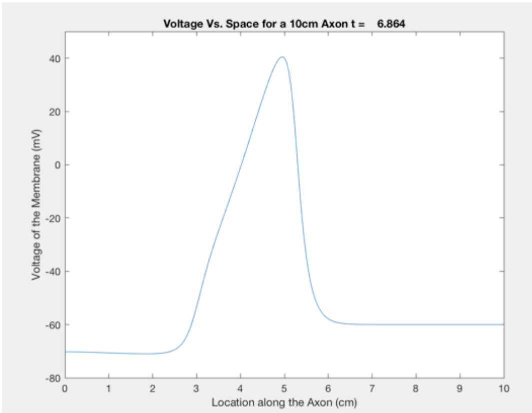

Fig. 20: Action Potential propagation when Ri=30Kohm/cm

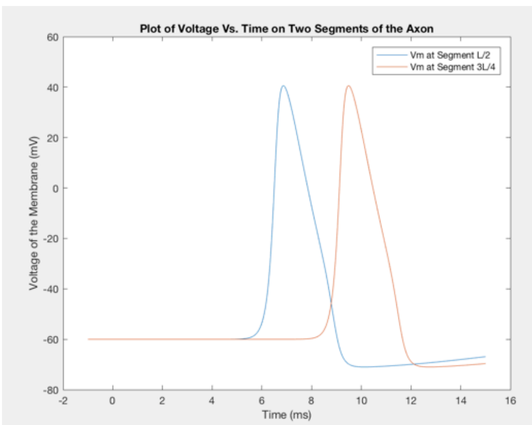

Fig. 21: Voltage vs Time when Ri=30Kohm/cm

In order to visualize the relationship between propagation velocity and ri, we constructed a plot which compares their values. It is shown below.

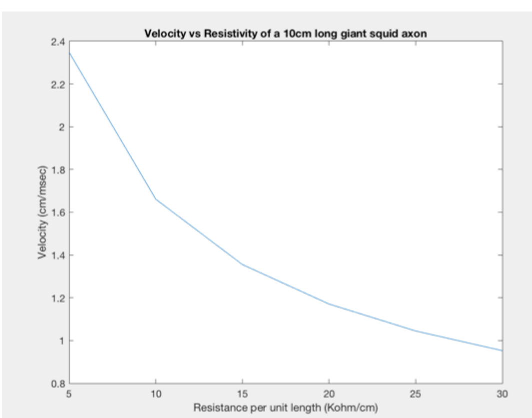

Fig. 22: Propagation velocity vs extracellular resistance

IV. Discussion

Our simulation successfully mimicked the propagation of an action potential down a giant squid axon, and from this model we were able to compute its velocity to be 0.9527cm/msec when ri was equal to 30Kohm/cm, 1.0447cm/msec when ri was equal to 25Kohm/cm, 1.1708cm/msec when ri was equal to 20Kohm/cm, 1.0447cm/msec when ri was equal to 15Kohm/cm, 1.3554cm/msec when ri was equal to 10Kohm/cm, 1.6611cm/msec when ri was equal to 10Kohm/cm, and 2.3481cm/msec when ri was equal to 5Kohm/cm. These results depict an inverse relationship between resistivity and velocity, and the speed of the propagating action potential decreased non-linearly as Ri was increased. The upstroke velocity (dV/dtmax) did not differ between the membrane and propagated action potentials, which was expected because the membrane potential in a cell is dependent on the presence or absence of an action potential. Therefore, the membrane potential will change at the same rate as the rate of the potential causing that change. Our values for ri=5Kohm/cm were comparable to the true velocity of a giant squid action potential (2.5 cm/msec in a 0.5mm axon), suggesting that our model is relatively accurate. The accuracy seen in our results highlights the main advantage of the Hodgkin-Huxley model, which is its ability to reflect the general behavior of a variety of cell types under a wide range of conditions.

V. Appendices

Dylan Kennedy was responsible for generating the majority of our MATLAB script, along with its plots. Dylan Young was responsible for writing the report, along with conducting the dimensional analysis associated with the units of ri+ro. In order to run the our script, one must simply copy the code posted below and click run.This will display the graph of a simulated action potential along the length of a 10cm long giant squid axon, as well as a comparison of the action potential over time at two segments of the axon. Comments are provided for each line if one wishes to change any of our set parameters, and the value for ri can be changed before every iteration of our code. This code is provided below, along with the dimensional analysis for the units of ri and ro.

A. Dimensional Analysis

Let $x = (r_i + r_o)$:

(1) $\frac{1}{x} \times \frac{1}{cm} \times \frac{mV}{cm^2} = \frac{\mu A}{cm^2}$

(2) $\frac{1}{x} \times \frac{mV}{cm} = \mu A$

(3) $\frac{1}{x} = \frac{\mu A}{mV} \times \frac{cm}{1}$

Let $\frac{\mu A}{mV} = \frac{1}{K\Omega}$:

(4) $\frac{1}{x} = \frac{cm}{K\Omega}$

(5) $x = \frac{K\Omega}{cm}$

Therefore:

$$(r_i + r_o) = \frac{K\Omega}{cm}$$

Fig. 23

B. MATLAB Script

```matlab
% BME 307: 1D propagation of the HH action potential along an axon
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Set up Time and Space Dimensions
%%clear all previous variables
clear all;
plotInterval = 10;

zStart = 0; % set up initial location, cm
zEnd = 10; % set up final location, cm
zLength = zEnd; %length of axon, cm
dz = 0.05; %change in z
tStart = -1.000 ; % start time, millisec
tEnd = 15.000 ; % end time, millisec


%%% Set initial variables
n_init = 0.31768; %initial value of n gate
m_init = 0.05293; %initial value of m gate
h_init = 0.59612; %initial value of h gate
EK = -72.1 ; % potassium nernst potential, mV
ENa = 52.4 ; % sodium Nernst potential, mV
EL = -49.187 ; % leak nernst potential, mV
Cm = 1.00; % microfarads
Vrest = -60 ; % resting potential, mV
gK_max = 36.0 ;% potassium saturation conductance, mS/cm^2
gNa_max = 120 ; % sodium saturation conductance, mS/cm^2
gL_max= 0.3 ; % leak saturation conductance, mS/cm^
ro = 0;
ri = 30;
a = 0.025;
D = 1/(2*a*pi*(ri+ro)*Cm);
watchSeg1 = 100;
watchSeg2= 150;
deltaT = (0.75*0.5*dz*dz)/D

% Divide the axon into several small segments
nSeg = ceil(zLength/dz) ; % number of segments on the axon, no units
nStep = ceil((tEnd-tStart)/deltaT) ; % number of time steps

% Create the stimulus current
Jstim = zeros(nSeg, 1) ; % stores the stimulus current
Jstim(2:4) = 50 ; % apply 50 uA/cm2 to segments 2,3, and 4
StimDur = 4;

% Create arrays to store all variables along the axon
Vm = Vrest*ones(nSeg, 1) ; % stores present Vm along the axon
Vm_new = zeros(nSeg, 1) ; % stores "future" Vm along the axon
n = n_init*ones(nSeg, 1) ; % stores gate n along the axon
m = m_init*ones(nSeg, 1) ; % stores gate m along the axon
h = h_init*ones(nSeg, 1) ; % stores gate h along the axon
n_new = n_init*ones(nSeg, 1) ; % stores gate n along the axon
m_new = m_init*ones(nSeg, 1) ; % stores gate m along the axon
h_new = h_init*ones(nSeg, 1) ; % stores gate h along the axon
dV_dt = zeros(nSeg, 1);
dn_dt = zeros(nSeg, 1);
dm_dt = zeros(nSeg, 1);
dh_dt = zeros(nSeg, 1);
d2vt = zeros(nSeg, 1);

% Use a matrix to store Vm for plotting
plot_Vm = zeros(nStep, nSeg) ; % save Vm for plotting
plot_Vm1 = zeros(nStep, 1);
plot_Vm2 = zeros(nStep, 1);
plot_dvdt1 = zeros(nStep, 1);
plot_dvdt2 = zeros(nStep, 1);

tNow = tStart ;
for iStep = 1:nStep % time loop: compute everything at each segment
if( 0<=tNow && tNow<StimDur ) % start stimulus current at tNow=0
    Jm = Jstim ;
    else % stop stimulus when tNow = StimDur
    Jm = zeros(nSeg, 1) ;
end
% Compute ion currents & stimulus current along the axon
for iSeg = 2:nSeg-1 % exclude the two end segments (boundaries)
JK = gK_max*n(iSeg)*n(iSeg)*n(iSeg)*n(iSeg)*(Vm(iSeg)-EK) ; % deltaVm = ((Jm - JNa - JK - JL)/Cm) * deltaT
JNa = gNa_max*m(iSeg)*m(iSeg)*m(iSeg)*h(iSeg)*(Vm(iSeg)-ENa) ;
JL = gL_max*(Vm(iSeg)-EL) ;
```

```
Jion = JK + JNa + JL;
% Compute gates' opening and closing
    rates
[alpha_n, beta_n] = getthe_n_rates(Vm(
    iSeg)); % get potassium activation
    rates
[alpha_m, beta_m] = getthe_m_rates(Vm(
    iSeg)); % get sodium activation rates
[alpha_h, beta_h] = getthe_h_rates(Vm(
    iSeg)); % get sodium inactivation
    rates

%Store values of Vm in the time based
    arrays for calculating velocity

dV_dt(iSeg) = ((Jm(iSeg) - Jion)/Cm) + (D
    .*((Vm(iSeg-1)-2.*Vm(iSeg)+Vm(iSeg+1))
    /(dz.*dz))) ;
dn_dt(iSeg) = (alpha_n*(1-n(iSeg))-beta_n
    *n(iSeg));
dm_dt(iSeg) = (alpha_m*(1-m(iSeg))-beta_m
    *m(iSeg));
dh_dt(iSeg) = (alpha_h*(1-h(iSeg))-beta_h
    *h(iSeg));

if iSeg == watchSeg1
    plot_Vm1(iStep) = Vm(iSeg);
    plot_dvdt1(iStep) = dV_dt(iSeg);
elseif iSeg == watchSeg2
    plot_Vm2(iStep) = Vm(iSeg);
    plot_dvdt2(iStep) = dV_dt(iSeg);
end

%Record the activation times for the two
    segment sections
if Vm(iSeg)>=-30 && iSeg == watchSeg1
    tactivate1=tNow;
end
if Vm(iSeg)>=-30 && iSeg == watchSeg2
    tactivate2=tNow;
end
end % for iSeg
% Update all the state variables along
    the axon
% again exclude the boundaries
Vm_new = Vm + deltaT.*dV_dt ;  %new Vm
n_new = n + (deltaT*dn_dt) ; % new n gate
m_new = m + (deltaT*dm_dt) ; % new m gate
h_new = h + (deltaT*dh_dt) ; %new h gate

 % for iSeg
tNow = tStart + iStep*deltaT ; % step
    forward in time
Vm(2:nSeg-1) = Vm_new(2:nSeg-1) ; % new
    Vm becomes present Vm
n(2:nSeg-1) = n_new(2:nSeg-1) ; % new n
    becomes present n
m(2:nSeg-1) = m_new(2:nSeg-1) ; % new m
    becomes present m
h(2:nSeg-1) = h_new(2:nSeg-1) ; % new h
    becomes present h
% Update the boundaries: no-flux
Vm(1) = Vm(2) ; Vm(nSeg) = Vm(nSeg-1) ;
% periodic: Vm(1) = Vm(nSeg-1) ; Vm(nSeg)
    = Vm(2) ;
% Save Vm along the axon at this time
    step
plot_Vm(iStep,1:nSeg) = Vm(1:nSeg) ; %
    After script runs correctly!
plot_time(iStep) = tNow ;
% Now plot the present values of Vm; this
    could
% instead be done below, outside of the
    main time loop
if mod(iStep, plotInterval) == 0
    plot( dz*(0:nSeg-1), plot_Vm(iStep,:)
        ) ; % plot Vm vs space
    axis([zStart zEnd -80 50]);
    ylabel('Voltage of the Membrane (mV)
        ');
    xlabel('Location along the Axon (cm)
        ');
    title(sprintf('Voltage Vs. Space for
        a 10cm Axon t = %8.3f', tNow));
    drawnow;
end % if plotInterval

end % for iStep

% Post-processing: more plots, dV/dt &
    APD computations, etc, happen here...
threefourths = 7.5 ; %cm
onehalf = 5 ; %cm
plot(plot_time, plot_Vm1, plot_time,
    plot_Vm2);
ylabel('Voltage of the Membrane (mV)');
xlabel('Time (ms)');
title('Plot of Voltage Vs. Time on Two
    Segments of the Axon');
legend('Vm at Segment L/2','Vm at Segment
    3L/4');

%Calculation of propagation velocity
velocity = (threefourths-onehalf)/(
    tactivate2-tactivate1) %cm/msec
[Maxdvdt1,Idvdt1] = max(plot_dvdt1)
[Maxdvdt2,Idvdt2] = max(plot_dvdt2)
[Maxvm1,Ivm1] = max(plot_Vm1)
[Maxvm2,Ivm2] = max(plot_Vm2)

%get_n_rates
```

```
function[alpha_n, beta_n]=getthe_n_rates(
    Vm)
if (Vm > -50.01) && (Vm < -49.99)
     alpha_n = 0.1;
     beta_n = 0.125*exp(-0.0125*(Vm+60));
         % ms^-1
else
     alpha_n = ((-0.01*(Vm+50))/(exp
         (-0.1*(Vm+50))-1)); % ms^-1
     beta_n = 0.125*exp(-0.0125*(Vm+60));
         % ms^-1
end
end

%get_m_rates

function[alpha_m, beta_m]=getthe_m_rates(
    Vm)
if (Vm > -35.01) && (Vm < -34.99)
     alpha_m = 1; % ms^-1
     beta_m = 4*exp(-(Vm+60)/18); % ms^-1
else
     alpha_m = ((-0.1*(Vm+35))/(exp(-0.1*(
         Vm+35))-1)); % ms^-1
     beta_m = 4*exp(-(Vm+60)/18); % ms^-1
end
end

%get_h_rates

function[alpha_h, beta_h]=getthe_h_rates(
    Vm)
alpha_h = 0.07*exp(-0.05*(Vm+60)); % ms
    ^-1
beta_h = 1/(exp(-0.1*(Vm+30))+1); % ms^-1
end
```

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VII. References

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