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CLAB #2: Ion Channels

1. Problem #1

```
clc; clear all; close all;
load actdata;
load deactdata;

% Problem 1

V_act=(actdata(1,2:end)); % isolate the voltage protocol
t_act=(actdata(2:end,1)); % isolate the time
I_act=(actdata(2:end,2:end)); % isolate the currents
figure(1);
for x = 1:24;
  plot(t_act,I_act(:,x)); % plot I vs t for all voltages on same graph
  hold on;
end
title('Figure 1: Activation Current (nA) vs Time (ms) at Voltages from -150 to 80 mV');
xlabel('Time (ms)');
ylabel('Activation Current (nA)');
hold off;

V_deact=(deactdata(1,2:end)); % isolate the voltage protocol
t_deact=(deactdata(2:end,1)); % isolate the time
I_deact=(deactdata(2:end,2:end)); % isolate the currents
figure(2);
for x = 1:31;
  plot(t_deact,I_deact(:,x));
  hold on;
end
title('Figure 2: Deactivation Current (nA) vs Time (ms) at Voltages from -250 to 50 mV');
xlabel('Time (ms)');
ylabel('Deactivation Current (nA)');
```

Activation data:

The values between -150 and 80 seem to be relative to the Nernst potential for Potassium. The first figures show that potentials below 0 are producing negative currents (this normally would only happen for potentials below that of Ek…. So it must be that 0 is a referenced Ek). The eqns given seem to indicate this would be the case.

During the time the voltage is held at a value (ie: <20ms) the potassium ions flow into or out of the cell according to the equilibrium potential for potassium. They flow out in magnitude proportional to the number of open channels which depends on the voltage (it's a voltage gated channel) where lower voltages produce fewer open channels. Ie: for membrane voltages held above the value of Ek, the potassium flows outward (positive deflection) but for values below that of Ek, the potassium flows inward (negative deflection) but these are weaker since fewer channels are open. Since the membrane is held constant at a voltage (between -150 and 80) there is no actual membrane repolarization or depolarization occurring during the 20ms period… however the induced currents are attempting to counter the effects of the voltage maintenance to move the potential toward Ek. For the membrane voltages less than Ek, the potassium flow is depolarizing… whereas for membrane voltages greater than Ek, the potassium flow is repolarizing.

After 20ms, the potassium ions are flowing into the cell (negative deflection) attempting to depolarize back toward Ek. The tail currents quickly return to 0 because when voltage maintenance is removed… the voltage gated channels are closing. Ions only flow while the channel is open, and that only happens at higher voltages. Therefore the channel current will stop abruptly, only continuing as long as the channel can remain open from some faint left over voltage from the maintenance (which quickly goes away). The initial amplitude of the tail current provides a means of comparing the amount of potassium conductance activated during the step.

Deactivation

In the first 20ms, when Vm is held at 50mV, the voltage gated channels are all open and Vm is greater than Ek… so the potassium ions flows outward to attempt to repolarize the membrane. At the lowest dropped voltage the potassium ions flow into the cell, attempting to depolarize the membrane (but they quickly drop off because of the closing channels (without voltage to open them). The highest voltages produce a net outward ion flow congruent with the initial conditions during the 20ms period. Membrane voltage is positive and therefore the channels are open permitting flow. The flow is outward to attempt to repolarize membrane potential toward Ek.

2. **Problem #2:**

```
%problem 2
%find maximum(negative)tail current at each activation voltage
[rows,cols] = size(I_act);
P_o = zeros(1,cols);
for i=1:cols
    P_o(i) = min(I_act(:,i));
end
P_max = min(P_o);
normalized_Prob = P_o/P_max;
figure;
hold all;
plot(V_act,normalized_Prob,'o');
title('Best Fit: Po/Pmax vs. Voltage (mV)');
ylabel('normalized Probability (Po/Pmax)'); xlabel('Voltage (mV)');
%BestFit:%General model:
%       f(x) = (a_o.*exp(z_a.*x))./((a_o.*exp(z_a.*x))+(b_o.*exp(-z_b.*x)))
% Coefficients (with 95% confidence bounds):
       a_o =    -0.04768;  %(-1.471e+05, 1.471e+05)
       b_o =    -0.01967;  %(-6.07e+04, 6.07e+04)
       z_a =    -0.08504;  %(-1.787e+04, 1.787e+04)
       z_b =       0.142;  %(-1.787e+04, 1.787e+04)
% remember that z_a and z_b here include the factors F/RT... must remove
% them obtain actual z_a and z_b
x = V_act;
bestFit = (a_o.*exp(z_a.*x))./((a_o.*exp(z_a.*x))+(b_o.*exp(-z_b.*x)));
plot(x,bestFit); grid on;
```

A researcher would look at the behavior of the current through channels immediately following a voltage drop because if all the tail currents occur at the same dropped voltage… the initial amplitude of the tail current provides a means of comparing the amount of potassium conductance activated during the step. This process requires no assumption that channels obey ohms law…. And since the channels deviate slightly from ohms law, this is a good technique. It allows us to quantify conductance of the potassium channels.

Problem 2.b:

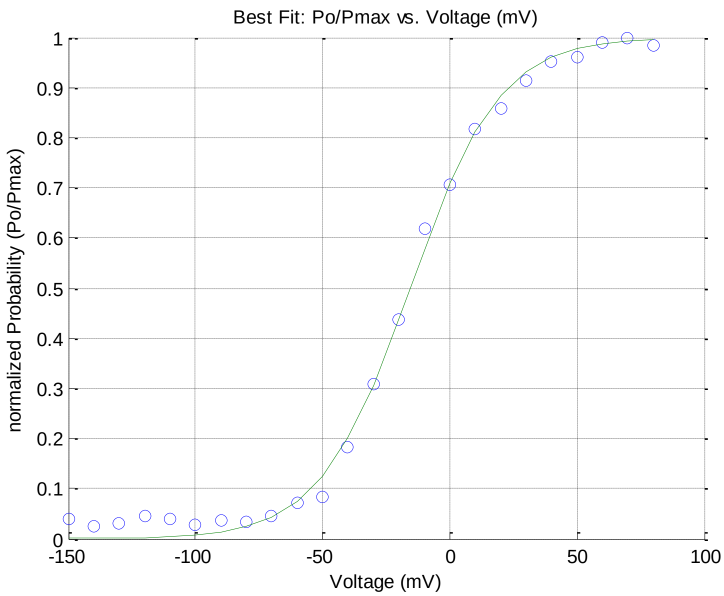


Figure 1: Plot of open probability vs voltage for each of the activation currents, with best fit.

The best fit eqn for P_o was found to be as follows:

```
%General model:
%       f(x) = (a_o.*exp(z_a.*x))./((a_o.*exp(z_a.*x))+(b_o.*exp(-z_b.*x)))
% Coefficients (with 95% confidence bounds):
       a_o =     -0.04768;  %(-1.471e+05, 1.471e+05)
       b_o =     -0.01967;  %(-6.07e+04, 6.07e+04)
       z_a(F/RT) =     -0.08504;  %(-1.787e+04, 1.787e+04)
      ∴z_a = 0.6856
       z_b(F/RT) =        0.142;  %(-1.787e+04, 1.787e+04)
      ∴z_b = 0.4939

% Goodness of fit:
%   SSE: 0.01183
%   R-square: 0.997
%   Adjusted R-square: 0.9965
%   RMSE: 0.02432
```

Normalized open probability P_o/P_{max} relates to the conductance through the channel because.... The graph of open probability over a range of voltages shows the voltage dependence of the open probability of the channel. Since the open probability of the channel supplies a means of comparing the likelihood of the channel being open (and therefore conducting)… it factors into the conductance of the channel. A channel could be a good conductor, but if it never opens it won't conduct.

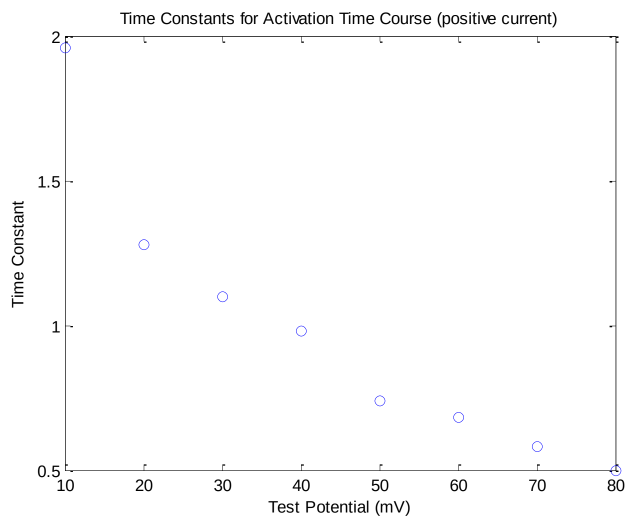
3. Problem #3:

```
%problem 3:
%calculate what 63% of the max value is, then find value in current vector
%that is >= to that and get its index.... then get that indice's value out
%of the time vector.... remember to subtract off the bit of time before any
%rise

% index_10mV = find(10==V_act);
% index_80mV = find(80==V_act);


tau(:,1)=V_act(10<=V_act)';  %make the first column of tau matrix the voltage values
for i=find(10==V_act):find(80==V_act)
    I_at_tau = 0.63*max(I_act(:,i)); %the value of the current at the time constant... ie 63% of
max
    index_1 = find(I_act(:,i) > 0.0001 ,1);
    index_2 = find(I_act(:,i) >= I_at_tau ,1);
    tau(i+1-find(10==V_act),2) = t_act(index_2)-t_act(index_1);  %place the time constant into
the array next to its associated voltage
end

figure;
plot(tau(:,1),tau(:,2),'o');
title('Time Constants for Activation Time Course (positive current)');xlabel('Test Potential
(mV)');ylabel('Time Constant');
```



Problem 3.2: The graph shown uses a best fit with an eqn that is a combination of 2 exponentials. Given that this is a single channel model, and there are 2 exponential eqns, I would expect 2 states. So the two-state model for this channel seems valid.

Figure 2: Rising time constants as a function of voltages that produce positive current flow.

4. Problem #4:

```
%problem 4
for i=0:7
    I(i+1) = max(I_act(:,i+17));
end
my_N_gamma = I./(normalized_Prob(17:24).*V_act(17:24));
avg_Ngamma = mean(my_N_gamma);
display('My N*Gamma is: ');
display(avg_Ngamma);

% %
```

```
% Problem 4
% Use these parameters for the normal model
a_o=.345;
b_o=.1865;
z_a=.02564;
z_b=.01847;
% make changes to a_o and/or b_o here to test the model
a_o_new=.345;
b_o_new=10*.1865;

time(1:1000) = 0:.02:19.98; % Create a time vector in ms stepping by .1 ms
time(1001:1525)=0:.02:10.48; % restart time at the repolarization moment
timeplot=0:.02:30.48; % this is a continuous time vector for use in the plot
Va=-150; % starting value for activation voltage
Ngamma = 0.2007;

figure(8);
% I don't recommmend that you change anything in this for loop.
for p=1:24
    % First create the voltage protocol
    Vhold=-80; % resting voltage
    Vrepol=-80; % repolarization voltage

    Vpro(1:40)=Vhold; % first part of voltage protocol: resting voltage
    Vpro(41:1000)=Va; % middle of protocol; activation voltage, which varies from -150 to 80
    Vpro(1001:1525)=Vrepol; % last part of voltage protocol: repolarization voltage

    alpha_model=a_o*exp(z_a*Vpro); % creates a new alpha using the voltage protocol
    alpha_new=a_o_new*exp(z_a*Vpro); % creates alpha using your modification

    beta_model=b_o*exp(-z_b*Vpro); % creates a new beta using the voltage protocol
    beta_new=b_o_new*exp(-z_b*Vpro); % creates beta using your modification

    tau_model = 1./(alpha_model+beta_model); % creates a tau for the model
    tau_new = 1./(alpha_new+beta_new); % creates modified tau

    Po_ss_model = alpha_model./(alpha_model+beta_model); % steady state Po for this model
    Po_ss_new = alpha_new./(alpha_new+beta_new); % creates modified Po steady state

    % the next lines create the open probability vector in both conditions at the activation
    % voltage only, which is (1-exp(t/tau)*Po_steadystate))
    Po_model(1:1000)=(1-exp(-time(1:1000)./tau_model(1:1000))).*Po_ss_model(1:1000);
    Po_model_new(1:1000)=(1-exp(-time(1:1000)./tau_new(1:1000))).*Po_ss_new(1:1000);
    % the next line creates Po at the repolarization voltage.
    % the initial condition of Po is taken to be the same as it was just
    % before the repolarization occurred
    % first create the initial conditions
    Po_initial = Po_model(1000)*((exp(-time(1001:end)./tau_model(1001:end))));
    Po_initial_new = Po_model_new(1000)*((exp(-time(1001:end)./tau_new(1001:end))));

    % the open probability now needs to have initial conditions
    Po_model(1001:1525)=((1-exp(-
time(1001:end)./tau_model(1001:end))).*Po_ss_model(1001:end))+Po_initial;
    Po_model_new(1001:1525)=((1-exp(-
time(1001:end)./tau_new(1001:end))).*Po_ss_new(1001:end))+Po_initial_new;

    I_model(:,p)=Ngamma*Po_model.*Vpro; % this creates the current data
    I_model_new(:,p)=Ngamma*Po_model_new.*Vpro;

    plot(timeplot,I_model(:,p),'b-',timeplot,I_model_new(:,p),'g-');
    hold on
    Va=Va+10; % advance to the next voltage protocol parameter
end

% change the title here to reflect your changes
title('Figure 8: Test of model parameters with beta = 10x normal beta:');
xlabel('Time (ms)');
ylabel('Activation current (nA)');
legend('Normal', 'Modified');

display('Their N*Gamma is: ');
```

```
display(Ngamma);
```

The Ngamma provided was **0.2007μS**…. the Ngamma I calculated was **0.1977 μS**, which is only 1.49% error off.

α_o seems to be linked with the activation time constant, whereas β_o seems to be linked with the inactivation time constant. Increasing α_o decreases $\tau_{activation}$ and increases magnitude of activation steady state but greatly decreases magnitude of deactivation steady state (see figure 3). Decreasing α_o increases $\tau_{activation}$ and decreases magnitude of activation steady state but increases deactivation steady state (see figure 4).

Increasing β_o decreases magnitude of activation steady state increases magnitude of inactivation steady state, and decreases $\tau_{in\text{-}activation}$ (see figure 5). Decreasing β_o increases magnitude of activation steady state, greatly decreases magnitude of deactivation steady state and increases $\tau_{in\text{-}activation}$ (see figure 6).

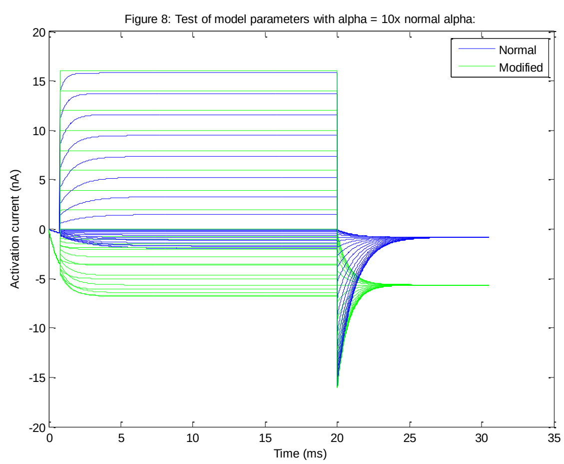


Figure 3: Increased alpha

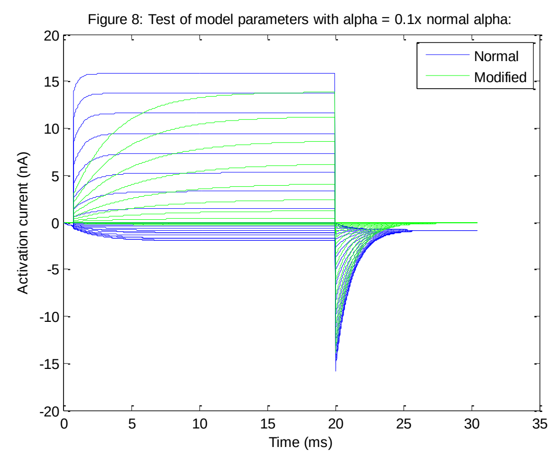


Figure 4: Decreased alpha

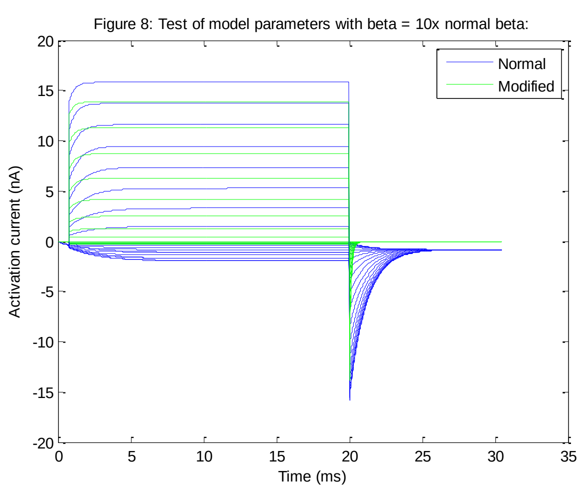


Figure 5: Increased Beta

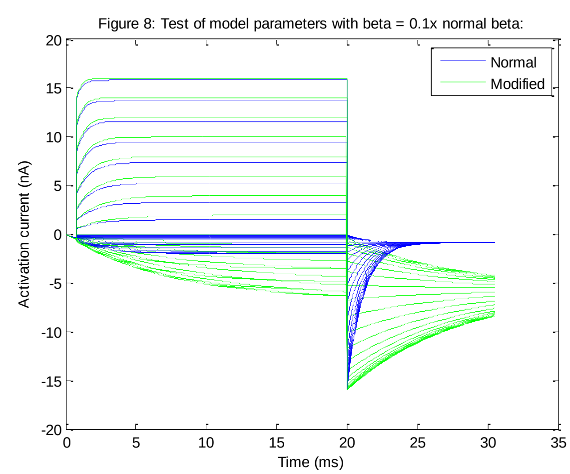


Figure 6: Decreased Beta

5. **Problem #5**

$dO/dt = \alpha C - \beta O$, where $C = 1 - O$

$dO/dt = \alpha(1-O) - \beta O = \alpha - (\alpha+\beta)O$

For general form $dX/dt = A - Bx$

general soln $1/(A-Bx)dx = dt$

integrate for $\ln(A-BX) + C = -Bt$

$\therefore dX/dt = A - BX = Ce^{-Bt}$

$\therefore dO/dt = (\text{constant})e^{-(\alpha+\beta)t} = \alpha - (\alpha+\beta)O$

$\therefore O = (\alpha/(\alpha+\beta)) - (\text{constant})e^{-(\alpha+\beta)t}$

i. O @ t=inf goes to zero

ii. O @ t = 0 goes to ($O_{infinity}$ – constant) because the exponent goes to 1

iii. $\therefore$ Constant = $O_{infinity} - O_0$

$O(t) = O_{inf} - (O_{inf} - O_0)e^{-t/tau}$ where $tau = 1/(\alpha+\beta)$ [this is the time constant for the system to relax toward equilibrium P_o

$\alpha = \alpha_o \exp(z_\alpha FV/RT)$ & $\beta = \beta_o \exp(-z_\beta FV/RT)$

Since C+O=1, $P_o = O/(C+O) = O$

$\therefore P_o = \alpha/(\alpha+\beta)[1-e^{-t/tau}]$, where $tau = 1/(\alpha+\beta)$

Simplify $P_{inf} = \alpha/(\alpha+\beta)$

$\alpha/(\alpha+\beta) = 1/[1+(\beta_o \exp(-z_\beta FV/RT)/\alpha_o \exp(z_\alpha FV/RT))]$

$\alpha/(\alpha+\beta) = 1/[1+\beta_o/(\alpha_o \exp((z_\alpha+z_\beta)(FV/RT)))]$

Substitute

Answer…. $\mathbf{P_o = 1/(1+(\beta_o/\alpha_o)\exp[-(z_\alpha+z_\beta)FV/RT])\ [1-\exp(t/tau)]}$

Discussants:

Satish, Paras Vora, Lauren Bedell, Maeve Woeltje, Mathew Everett