2D1435 Neuronnäts- och biomodellering
2D1436 Modellering av cellbiologiska processer
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Name: GK date: Sign:

Lab 2: Membrane potential and stochastic, voltage dependent ion channels

In this exercise you will investigate how the membrane potential of a cell is determined and how voltage gated ion channels operate. The goal of the lab is to get you acquainted with

- The laws of ion movement along concentration and potential gradients and across cell membranes, through leakage and voltage gated ion channels.

- The Goldmann-Hodgkin-Katz equation, which is used to calculate the resting potential of a cell, for single and multi-ion, conditions, as well as the currents through the cell membrane.

- The current-voltage differential equation of a single membrane compartment ("cell"). This is the basic component of multi-compartment cell models.

- How stochastic ion channels operate and how their voltage dependence may be modeled by the Hodgkin-Huxley equations.

- How a population of such channels can produce a sodium (Na) spike.

1. Tasks

You should implement most parts of this lab on your own. This includes the voltage and current versions of the Goldman-Hodgkin-Katz equation, a membrane compartment, and a stochastic Na channel.

At the examination of the lab, plots and questions marked with a "•" should be presented and answered respectively.

2. Lab environment

The tools you use in this lab are a matter of your own preference. You can use Matlab or the programming language of your choice for implementation and graphic output. Gnuplot is an easy to use Unix plot program for those who don't prefer Matlab.

Already from the start you should create a separate directory in which you run the lab.

3. The Goldmann-Hodgkin-Katz equation

Start by implementing the GHK voltage equation (JW 2.7.21) with three different ions, potassium (K), sodium (Na), and chloride (Cl):

$$V_{rest} = \frac{RT}{F} \log \frac{P_K \left[K^+ \right]_{out} + P_{Na} \left[Na^+ \right]_{out} + P_{Cl} \left[Cl^- \right]_{in}}{P_K \left[K^+ \right]_{in} + P_{Na} \left[Na^+ \right]_{in} + P_{Cl} \left[Cl^- \right]_{out}}$$

We assume a temperature of 293 °K. Use SI units in computations for the most part – though sometimes e.g. mV are handy units! Note that mM is the SI unit for concentration. The logarithm is base e, but you may convert it and use base 10 if you like. Values for the gas constant R and Faradays constant F are:

F = 96480 Coulomb/mol

R = 8.314 Joule/Kelvin/mol

	P (m/s)	C_{in} (mM)	C_{out} (mM)
K	4.00 10^{-9}	400	10
Na	0.12 10^{-9}	50	460
Cl	0.40 10^{-9}	40	5

- Calculate the membrane resting potential for the values in the table above of ion specific permeabilities (P) and intracellular (C_{in}) and extracellular (C_{out}) ion concentrations (in mol/l, "molar" designated by M).

- Interchange the C_{in} with C_{out} for all ions and calculate resting potential again. How does it relate to the above?

- What is the resting potential if only the K permeability is non-zero?

- What is the resting potential if only the Na permeability is non-zero?

Next, implement the GHK current equation of an ion species (JW 2.7.17):

$$I = \begin{cases} PzF(C_{in} - C_{out}) & if\ V = 0 \\[2em] PzF\xi\left[\dfrac{C_{in} - C_{out}e^{-\xi}}{1 - e^{-\xi}}\right] & otherwise \end{cases}$$

where

$$\xi = \frac{zVF}{RT}$$

Return to the same conditions as in the table above. Note that the currents carried by the individual ions should be calculated separately and then summed to get the total current.

- What is I_m at a V_m of –70mV and 0 mV? What is the dimension of I_m?

- Calculate the I-V relationship of the membrane for the same conditions as above (use GHK-I and step V from –80 to 80 mV in steps of 5 mV). Draw the I(V) curve.

4.   A spherical membrane compartment

Now, a spherical membrane compartment will represent a cell or a small part of it. A reasonable diameter of a neuron soma is 100μm whereas a dendritic spine head might be only 1μm. We assume a membrane thickness of 100 Angstrom, and a specific capacitance (c_m) of 1 μF/cm^2 (0.01 F/m^2). Implement the current-voltage differential equation for a soma sized compartment (JW eqn. 3.1.1). Set up so that you can later change size to a spine head.

$$C_m \frac{dV_m}{dt} = I_K + I_{Na} + I_{Cl} + I$$

where I is injected current. The different ionic components of I_m are derived from GHK-I. Note that the current should be positive inwards, which is *opposite* to how GHK-I was first derived. Note also that the currents are in the unit A now.

- What is I_m at V_m = –50 mV?

- What is the total membrane conductance of the cell at –50 mV?

Integrate the current-voltage diff equation using the simple Euler forward method ($V_m(t+\Delta t) = V_m(t) + I_m/C_m \Delta t$).

A timestep of 0.1 ms will be fine. Set up to run a simulation of 50 ms, i.e. 500 timesteps, starting at $V_m(0) = -50$ mV.

- Plot the time course of V_m!

- Estimate by inspection an approximate value of τ_m during these conditions.

  Hint: $V(t)$ has the form of $e^{-t/\tau}$.

Now, during the simulation, change permeabilities P according to this table:

| time	P_K	P_{Na}
$0.000 <= t < 0.010$	$4.00\ 10^{-9}$	$0.12\ 10^{-9}$
$0.010 <= t < 0.015$	$4.00\ 10^{-9}$	$6.00\ *10^{-9}$
$0.015 <= t < 0.025$	$4.00\ 10^{-9}$	$0.12\ 10^{-9}$
$0.025 <= t < 0.030$	$40.0\ 10^{-9}$	$0.12\ 10^{-9}$
$0.030 <= t$	$4.00\ 10^{-9}$	$0.12\ 10^{-9}$

- Plot the time course of V_m under these conditions. Verify that your dynamic value for the potential of the peak and the potential towards the end of the simulation approaches that calculated from GHK_V in section 3!

Change the diameter of your compartment to 1 μm (spine head size).

- What, if any, difference do you see between the whole cell and the spine head with regard to e.g. I_m and $V_m(t)$?

5.   Stochastic, voltage dependent ion channels

You should now implement a voltage dependent stochastic sodium channel with inactivation. We start with a single gating particle with two states, open and closed. Such a particle switches between open and closed as:
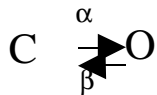
where α and β (dimension s^{-1}) are voltage dependent rates and $P(O \rightarrow C) = \beta \Delta t$ and $P(C \rightarrow O) = \alpha \Delta t$ (given small enough Δt).

Now, assume that for a Na-m particle α and β depend on transmembrane voltage V_m (in V) as:

$\alpha(V) = -10^5(V+0.035)/(e^{-(V+0.035)/0.010} - 1)$, $\alpha(-0.035) = 10^3$

$\beta(V) = 4000e^{-(V+0.060)/0.018}$

- Plot the curve for $m_\infty(V_m)$ and $\tau_{m_\infty}(V_m)$ for $V_m = -80$ to 80 with steps 10 mV.

Simulate your Na-m particle's dynamics. Again use a timestep of 0.1 ms and run for 0.050 s. The following Matlab code can be used to update a channel:

```
function next_state = next_state(state,alpha,beta,dt)
nch = size(state,2);
p01 = rand(1,nch);
alphadt = repmat(alpha,1,nch)*dt;
betadt = repmat(beta,1,nch)*dt;
next_state1 = (p01<alphadt) .* (state==0);
next_state0 = (p01<betadt) .* (state==1);
next_state = state + next_state1 - next_state0;
```

where `state` is the current state and `alphadt` is alpha(V)*timestep etc.

• Plot a curve of the state of such a particle for a period of 0.05 s at V_m = -80 to 80 with steps 10 mV.

The inactivation of the sodium channel is represented by an Na-h particle. Implement such a particle with:

$\alpha(V) = 12e^{-V/0.020}$

$\beta(V) = 180/(e^{-(V+0.030)/0.010} + 1)$

Compose a Na channel from three Na-m and one Na-h particle (a m^3h sodium channel).

• Verify its voltage dependence by plotting a curve of the state of the channel for a period of 0.05 s at V_m = -80 to 80 with 10 mV steps.

6.   Voluntary exersice. A compartment with added voltage dependent sodium channels

In this last part of the lab you should add a number of these stochastic model Na-channels to your spine head sized membrane compartment and compare the results with the previous "passive" compartment (in 4. above). You should run 0.050 s with the leak permeabilities as in the table in section 3 but with some amount of current (pA) injected between 10 and 15 ms into the simulation. Use 40 channels. The maximum (open) conductance of one Na channel is assumed to be 1 pS. Remember to use the equilibrium potential E_{Na} as calcuated in section 3.

Hint 1: Look at the example code enclosed. An ASCII file is found in the /info/biomodYY/ directory.

Hint 2: To save time, run shorter simulations while testing things out.

Adjust the injected current and number of channels so that you get a Na spike during which the top potential reaches up to about 20 mV given the conditions above, the potential without channels not exceeding -30 mV.

• What is the minimal current of your system that gives a "spike"?

• Decrement the number of channels by 10 down to 0. Draw plots of V_m over time for each condition. Does it look like the peak amplitude of V_m changes linearly with the number of channels?

• Returning to you original number of channels, what happens if you increase the K-permeability by a factor of ten between 20 and 25 ms. Explain why it happens!

Now you are done, thank you!

```matlab
% Example of Matlab code for Section 6 (incomplete, see <...> below)

clear;
PK = 4e-9;
PNa = 0.12e-9;
PCl = 0.4e-9;
Kin = 400;
Kut = 10;
Nain = 50;
Naut = 460;
Clin = 40;
Clut = 5;

diam = 1e-6; %spine
area = pi*diam*diam;
cm = 1e-6/1e-4; % converted to SI from F/cm^2

Cm = cm*area;

nstep = 0.100/0.0001;

nch = <N:o Na channels>;
Sm1arr = zeros(nstep,nch);
Sm2arr = zeros(nstep,nch);
Sm3arr = zeros(nstep,nch);
Sh1arr = zeros(nstep,nch);
Tarr = zeros(nstep,1);
Varr = zeros(nstep,1);

Sm1arr(1,:) = 0;
Sm2arr(1,:) = 0;
Sm3arr(1,:) = 0;
Sh1arr(1,:) = 0;

ENa = <Reversal potential for Na>;
GVNa = 1e-12; % Single channel conductance
V = -0.0674;
```

```
Varr(1) = V;
IVNa = 0;
for i=2:nstep
    t = 0.0001*i;
    Tarr(i) = t;
    Varr(i) = V;
    if and(0.010<=t,t<0.015)
        Iin = <current injected>;
    else
        Iin = 0;
    end
    if and(0.020<=t,t<0.025)
        PK = 40e-9;
    else
        PK = 4e-9;
    end
    IVNa = 0;
    for n=1:nch
    Sm1arr(i,n) = chupdate(Sm1arr(i-1,n),Nam_alpha(V),Nam_beta(V),0.0001);
    Sm2arr(i,n) = chupdate(Sm2arr(i-1,n),Nam_alpha(V),Nam_beta(V),0.0001);
    Sm3arr(i,n) = chupdate(Sm3arr(i-1,n),Nam_alpha(V),Nam_beta(V),0.0001);
    Sh1arr(i,n) = chupdate(Sh1arr(i-1,n),Nah_alpha(V),Nah_beta(V),0.0001);
    IVNa = IVNa + <channel current contribution>;
    end
    V = V + <potential change>;
end
figure(4);
plot(Tarr,Varr);
axis([0 nstep*0.0001 -0.1 0.1]);
```