PROBLEM SET

OHM’S LAW: \[ V = I \times R \quad \text{or} \quad I = g \times V \]

NERNST EQUATION: \[ E_K = \frac{RT}{zF} \ln \left[ \frac{[K^+]_\text{OUT}}{[K^+]_\text{IN}} \right] \]

where at room temp., \(2.3(\frac{RT}{F}) \approx 59 \text{ mV}\) and \(\ln = 2.3 \times \log_{10}\)

GOLDMAN-HODGKIN-KATZ EQUATION:

\[ V_{\text{rev}} = \left( \frac{RT}{F} \right) \ln \left( \frac{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_i}{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_o} \right) \]

1) a. What two factors determine the resting membrane potential in neurons?

b. List the typical millimolar concentrations for Na\(^+\), K\(^+\), Ca\(^{2+}\), and Cl\(^-\) outside and inside the neuron. What are the typical concentration gradients for these ions (outside/inside)?

<table>
<thead>
<tr>
<th>ION</th>
<th><a href="mM">inside</a></th>
<th><a href="mM">outside</a></th>
<th>Gradient</th>
</tr>
</thead>
</table>

2) Fill in the blank or circle the correct answer: [Note that the “resting state” refers to a stable membrane potential in the absence of voltage clamp]

a. In the resting state, the neuronal membrane potential is in the range of ________ mV.

b. In the resting state, the most permeable ion across the neuronal membrane is _____.

c. In the resting state, the net ion flow across the neuronal membrane is **inward** / **outward** / **zero** (circle one).

d. In the resting state, there is no ion flow across the neuronal membrane – **True** or **False**? (circle one)

e. The Nernst equation describes equilibrium when the ____________ driving force is balanced by the ______________ driving force.
3) Circle the word that correctly completes the sentence describing the conventions for intracellular (or whole-cell patch clamp) voltage-clamp recording:

a. Current is defined as the direction of flow of (positive / negative) ions.

b. The current sensing electrode is the one (outside / inside) the cell.

c. Outward current, flowing away from the sensing electrode, is (positive / negative).

d. The voltage outside the cell is (zero / variable) mV (i.e., outside the cell is “ground”).

e. Inward current, which flows toward the sensing electrode, is (positive / negative).

f. Cl⁻ flowing into the cell is considered an (inward / outward) current.
We are recording the voltage difference across the neuronal membrane shown above, where the ground electrode (V = 0) is outside the cell.

a) Calculate $E_{Na}$, $E_K$, and $E_{Cl}$.

b) We replace Cl$^-$ with the impermeant anion gluconate (otherwise, concentrations/ions are as listed on previous page). The $V_m$ is now -80 mV. What is $P_{Na} / P_K$?

Hint: Use Goldman-Hodgkin-Katz equation, and multiply through top and bottom by $1 / P_K$.

c) Cl$^-$ is restored to the bathing solution (and gluconate removed) and a drug is added to the bathing solution, resulting in increased Cl$^-$ conductance (i.e., the number of Cl$^-$ permeable channels increases). Will the $V_m$ (equilibrium potential) become more or less negative than in the previous condition (b)?

d) Again, we replace Cl$^-$ with gluconate. Suddenly, the membrane permeability changes such that $P_{Na} / P_K$ is 25. In the “first jiffy”:

a. Which direction will current flow (outward or inward)?
b. Will $V_m$ be positive or negative once steady-state is achieved?
c. Calculate $V_m$ at steady-state.
5) Now we apply a voltage clamp to the solutions shown on the previous page. $V_{OUT} = 0$.

a) Give the equation which relates $I_K$ to $V_H$ (the holding potential). Do the same for $I_{Na}$ and $I_{Cl}$.

b) For $V_H = -100$ mV, which direction is the $K^+$ current flow (inward or outward)? Which direction is the $Cl^-$ current flow?

c) Sketch the I-V curve for a membrane containing only HCN channels.

d) Sketch the I-V curve for a membrane containing only outwardly rectifying $K^+$-selective channels.
7) The Hodgkin-Huxley equations predict the current response to rapid changes in membrane voltage, by modeling the gating of voltage-dependent Na+ and delayed rectifier K+ channels. The following questions are based on your lecture notes on the H-H equations.

a) The above plot shows the peak current-voltage (I-V) relationship for voltage-dependent Na+ channels (labeled curve, left panel) and the corresponding conductance vs voltage plot (right panel).
   i. For what range of test potentials is the Na+ channel I-V curve ohmic (linear)?
   ii. How does the Na conductance plot predict the ohmic region of the peak I-V curve?
   iii. Explain why the I-V curve shows negative slope conductance from -50mV to -25mV.

b) Explain briefly the relationship between Na channel gating and the 2 variables, voltage and time, based on the equation and plot shown below.

\[ C \xrightarrow{\alpha} C \xrightarrow{\beta} C \xrightarrow{\alpha} C \xrightarrow{\beta} O \]
7c) The plot below shows Na+ channel conductance vs. time in response to the test potentials indicated (starting from -100 mV holding potential).

![Graph of Na+ channel conductance vs. time](image)

i. Superimposed on these traces, roughly sketch the K+ channel conductance vs time; amplitude is relative (Hint: you may need to extend the time axis; these plots can be found in your lecture notes!)

ii. Based on these plots of Na and K channel conductance vs time for -2mV and +44mV (assuming you are starting from -100mV where channels are in the closed but fully activatable state), sketch the current responses to a sudden jump to a test potential of -2mV and a second sketch of a jump to +44mV, plotting current vs. time, for both Na+ and K+ channels. You will need to take into account the driving force for each current.
8) In neurons, depolarizing or hyperpolarizing current (I) causes a change in membrane voltage (V). The following figure shows neuronal voltage responses in the absence of functional HCN channels. For both (A) and (B), draw in the voltage responses expected when HCN channels are present, and indicate which situation is depolarizing and which is hyperpolarizing. In addition, describe briefly how the properties of HCN channels allow them to actively oppose changes in membrane voltage. Why is this function important for neurons?
9) Methods:
Patch electrodes contained (in mM): 130 K-gluconate, 10 NaCl, 2 Na2ATP, 0.3 NaGTP, 10 HEPES, and 0.6 EGTA, buffered to pH 7.4 and ~275 mOsm. Slices and outside-out patches were perfused with media of the following composition (in mM): 130 NaCl, 24 NaHCO3, 3.5 KCl, 1.25 NaH2PO4, 1 CaCl2, 3 MgSO4, 0.2 CdCl2, and 10 glucose and tetrodotoxin (TTX, 0.5-1 µM) saturated with 95% O2 and 5% CO2, pH 7.4. In A, the current recorded after jumping to test potentials ranging from -60 to +70 mV (10 mV increments) from a pre-pulse holding potential of -30 mV was subtracted from the total current recorded after jumping to test potentials of -60 to +70 mV (10 mV increments) from a pre-pulse holding potential of -110 mV, and the difference current at each test potential is plotted.

The figure and methods section above are from a voltage clamp study on a K current. Please do the following:

1) Plot the activation and inactivation curves for the currents.
2) What is the maximum conductance of this current?
3) Estimate the ½ inactivation voltage for the current.
4) Describe what type of K current this is.
5) How would you prove that this is the current that you think it is? (e.g. What is the definitive blocker of this channel?)