Excitable Cell Physiology Problem Set #2:
Membrane Potentials and the Voltage Clamp

Animal Physiology
Fall 2015

1. Assume that a cell is absolutely **impermeable to Na^+** at rest and it is **freely permeable to K^+ and Cl^-**. Ignore all other ions. Here are the concentrations of each ion:

$[K^+]_{in}$ = 240 mM $[K^+]_{out}$ = 10 mM

$[Cl-]_{in}$ = 15 mM $[Cl-]_{out}$ = 360 mM

$[Na^+]_{in}$ = 20 $[Na^+]_{out}$ = 145.6 mM

The temperature is 20^o C

Calculate: i) $E_K{}^+$; ii) E_{Na+} ; iii) resting E_m

$E_K{}^+$ = -58mV *log (240 mM/10 mM) = - 80 mV

$E_{Na}{}^+$ = -58mV* log (20 mM/145.6 mM) = + 50 mV

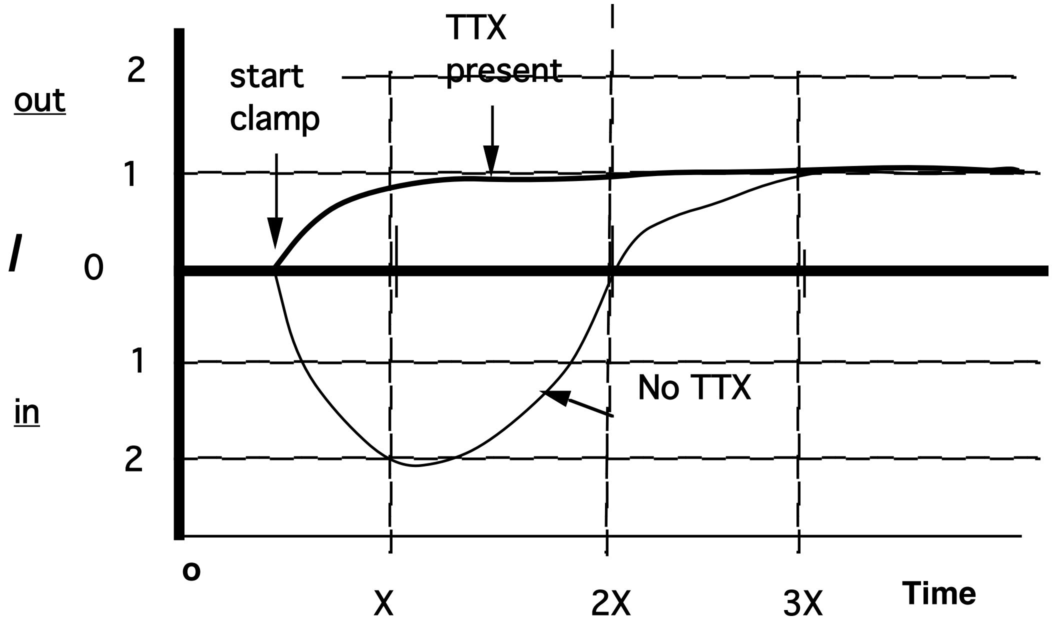
$$E_m = -58\text{mV} * \log(\frac{(P_K * [K^+]_{out} + P_{Cl} * [Cl^-]_{in})}{(P_K * [K^+]_{in} + P_{Cl} * [Cl^-]_{out})}$$

Note that Na^+ is not included in the Goldman since it is given that at rest there is no Na^+ permeability; likewise, since the permeabilities of both Cl^- and K^+ are equal then the equation becomes:

E_m = -58mV* log((240 mM + 360 mM) / (10mM + 15 mM) = -58 mV * log(600/25)

E_m = -80 mV

2. The cell is now voltage clamped at Em = - 40 mV -- a value well above its threshold. Here are the results of two runs, one with tetrodotoxin (TTX), and the other without:

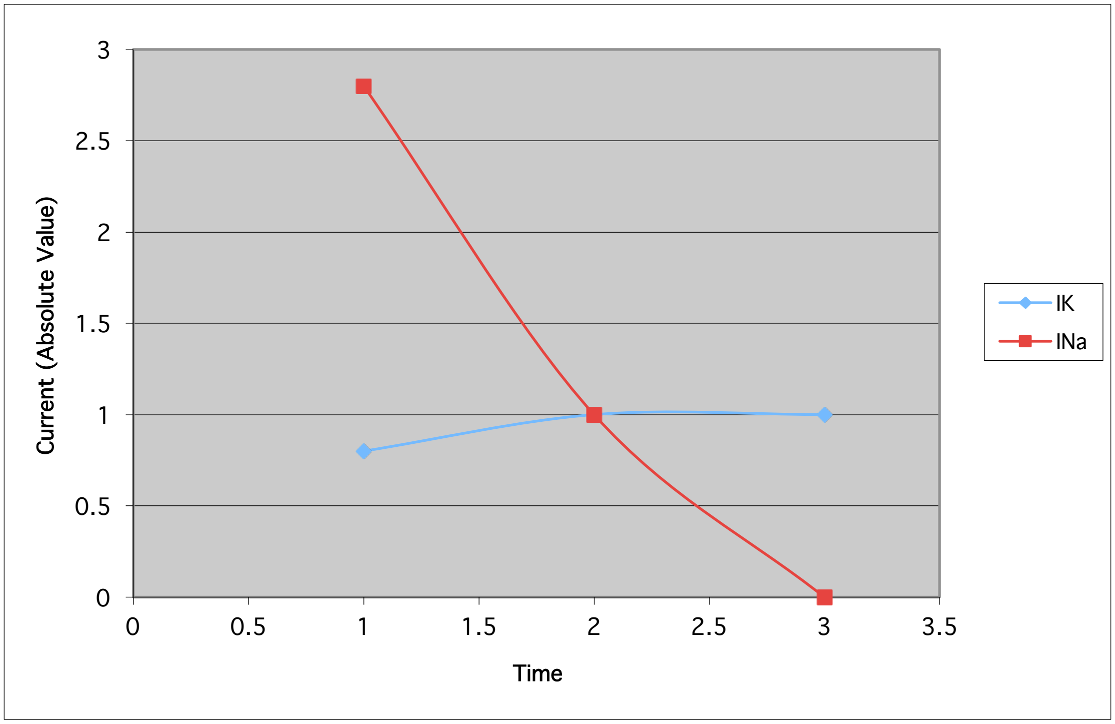


(a) Calculate G_K+ and G_{Na}+ for the three times x, 2x and 3x. (Don't worry about units).

The data above can be used to obtain I_{Na} and I_K. The "TTX" curve gives I_K and the difference between the TTX and no TTX curves gives I_{Na}. Here is a table of values picked off the graph:

Time	**TTX (I_K .) curve**	**no TTX (I_{Na} and I_K).**	**I_{Na}**
X	**0.8**	**-2.0**	**-2.8**
2X	**1.0**	**0**	**-1.0**
3X	**1.0**	**1.0**	**0**

A graph of the currents (given in absolute terms so that it is easier to compare their magnitudes) is on the next page:



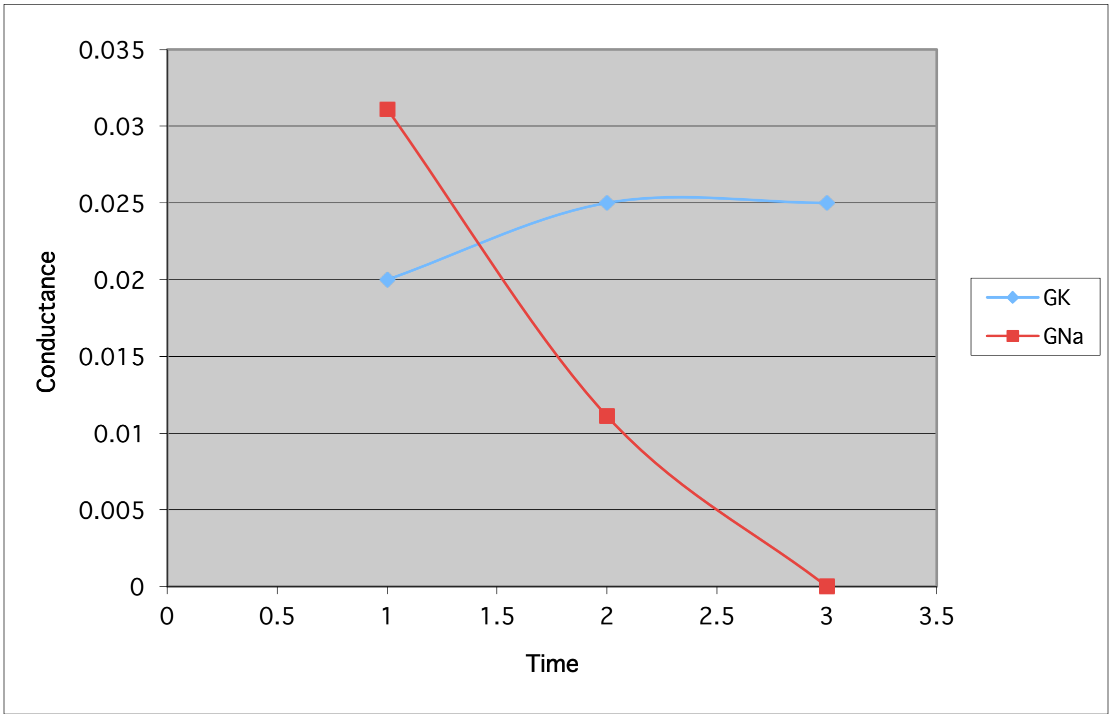
The general formula to find conductance is:

$G_{ion} = I_{ion} / (E_m - E_{ion})$ where E_{ion} is the Nernst potential for that ion and E_m is, in this case, the voltage clamp value (since it is the membrane potential). For instance, solving for G_{na} at time = X we find:

$G_{na} = -2.8 / (-40 - (+50)) = 2.8 / 90 = 0.0311$ "mhos" but notice we do not have real conductance units here since I have not provided units for current.

Here is a table with the appropriate values:

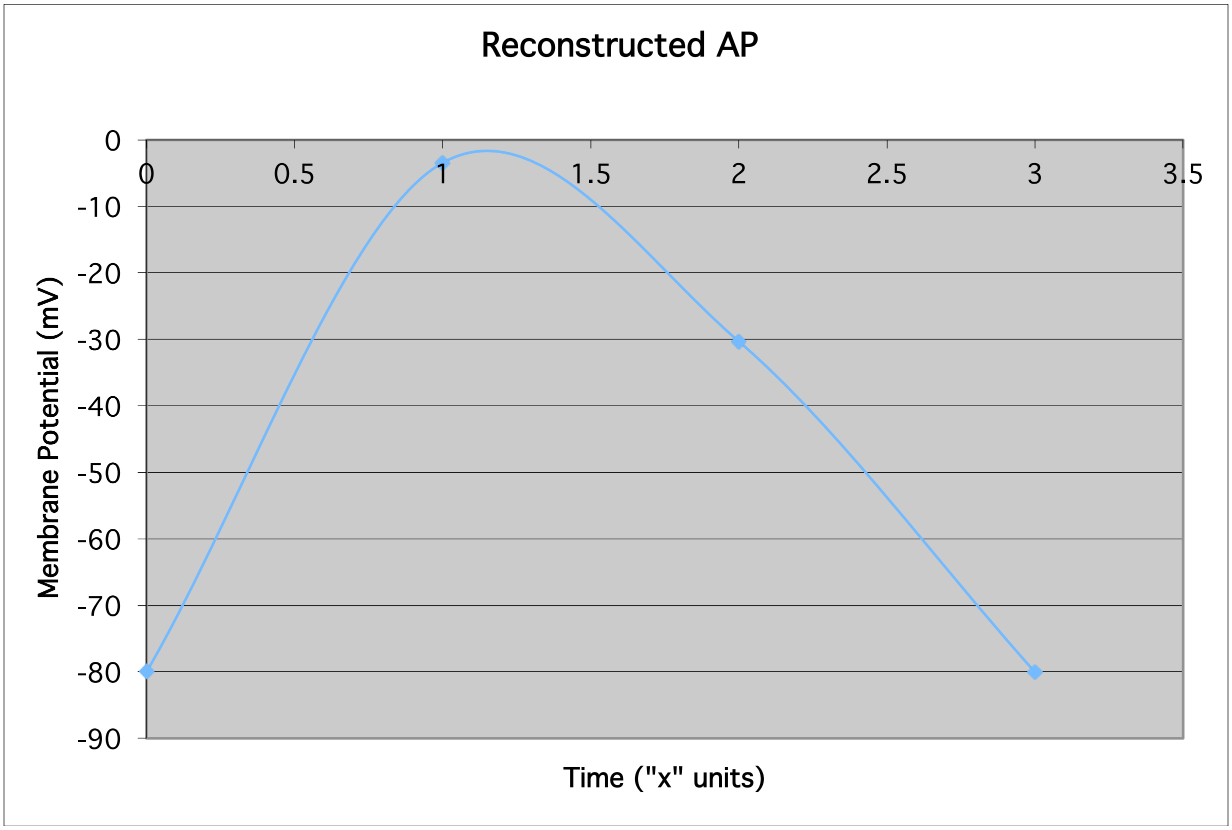
Time	**I_K**	**G_K**	**I_{Na}**	**G_{Na}**
X	**0.8**	**0.020**	**-2.8**	**0.0311**
2X	**1.0**	**0.025**	**-1.0**	**0.0111**
3X	**1.0**	**0.025**	**0**	**0**



(b) In this clamp experiment, what is the membrane capacitive current (I_C) at time 3x? Would it be the same in a non-clamped situation -- you need not give the actual value of I_C in this case, just the relative value? Explain your answer in no more than 3 sentences.

Zero -- since the membrane voltage is clamped, then there must be no charge flowing into or out of the membrane capacitor. Any additional charges being added or being removed would change the total number of charges stored in the capacitor and therefore the voltage measured across the capacitor (*i.e.*, across the plasma membrane) and this would change the potential since E = Q/C.

(c) Using the values you calculated for G above the ionic concentrations given in question 1; reconstruct an action potential (E_M vs. time) for the cell. Obviously, this refers to the unclamped condition. Note that you will make this reconstruction with a total of three values (talk about sampling!).



One additional note: don't fret that the voltage clamp data (see given data) seems to imply that all ions are impermeable and the membrane potential was zero in the moments before the clamp was set. This is an artifact of the way the experimental data are presented and needs not concern you; that is why in the graph above I have given the normal resting potential for this particular cell.