

## Lab #2 (Changing cell voltage *quickly*)

In this lab, we'll use BITSEY to run more simulations of single cells. This time, our goal will be to understand the short-term effects of how changing an ion-channel conductance affects  $V_{\text{mem}}$ .

Logistical note: like lab #1, you can (and should) work in pairs. However, please choose a different partner this time.

You can use the same BITSEY files as last week. This time, we'll be using and modifying the `setup_lab2()` and `setup_lab2b()` functions. You will notice that `setup_lab2()` is quite similar to `setup_lab1()`. How, then, is lab #2 different from lab #1?

In lab #1, we let the simulations run for many hours of simulated time. You probably noticed that the  $V_{\text{mem}}$  waveforms started at near 0 volts, then quickly "settled" to a value in less than a second, and then moved slowly (over tens of hours of simulated time) to their final values. In lab #2, we only care about those initial values – i.e., quasi steady state. Thus, all of our simulations will be just one second of simulated time. The wonderful thing about simulating in quasi steady state is that the simulations are all short 😊. This type of computation is how our brains work.

Thus, two very obvious changes for lab #2. First, we will run the simulations for only 1 second rather than the 100K seconds we used for lab #1 – so **python3 main.py lab2 2**. Second, `setup_lab2()` should definitely use the more stable (but much slower) numerical-integration algorithms (`p.adaptive_timestep = False`). Don't worry – the runs will still be fast.

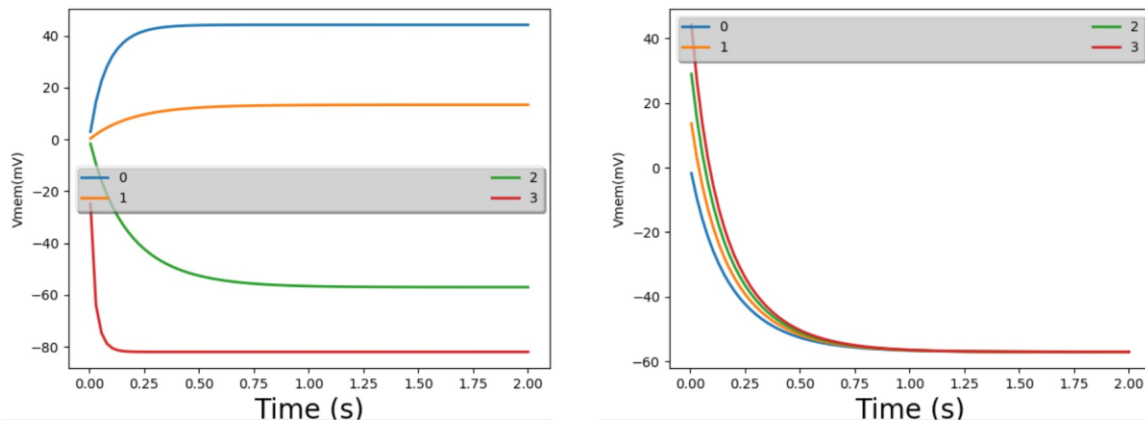
You will run two simulations for this lab. The first simulation run is to simply run `setup_lab2()` and save the graph of per-cell  $V_{\text{mem}}$ . It should show that the different values of ion-channel conductivity result in different quasi-steady-state  $V_{\text{mem}}$ .

After you do that, you have one more simulation run. We would like to understand how resilient this type of computing is to small changes in a cell's initial ion concentrations. To do that we will use a new function `setup_lab2b()`. It's partially written, and you will fill it in.

- Create 4 cells as usual, with no gap junctions, just as in `setup_lab2()`.
- All four cells should have the ion-channel diffusion constants from cell #2 of the first simulation – so a potassium diffusion constant of  $10.0\text{e-}18$  (i.e., `Dm_array[K,:]= 10.0e-18`), with the other ions remaining at their defaults. In `setup_lab2()`, this should have resulted in quasi-steady-state  $V_{\text{mem}} \approx -57\text{mV}$ .
- Leave cell #0 as a reference. For cells #1-3, perturb their initial [Na] *very slightly*, by an extra .005 moles/ $\text{m}^3$  for each successive cell. This should be just enough so that each cell has its time=0  $V_{\text{mem}}$  about 15mV higher than the previous cell (unlike lab #1, you will not compensate by changing another ion to restore charge neutrality). If you accidentally change the initial [Na] too much (and thus change the initial  $V_{\text{mem}}$  by more than about 0.5V), the simulator will not allow the simulation to run!

What happens as a result? The cells now have a different initial charge, and in fact no longer start out charge neutral. Their  $V_{\text{mem}}$  at time=0 will, therefore, now be nonzero, and in fact different in each cell. But does this affect the results at  $t=1$  second? Turn in the graph of  $V_{\text{mem}}$  for both `setup_lab2()` and `setup_lab2b()`.

The two graphs should look roughly like this:



Also, please answer the following questions:

1. Just as with lab #1, you can look at the data printed by *dump()* at the end of the simulation. In lab #1, we saw that for each individual ion, its flow through the pumps was equal and opposite to its flow through the ion channels. Is that still true? If not (and in fact it should not be), what claim can we make instead – that shows the system has reached quasi steady state?

Let's look at the data from `setup_lab2()`; 2b would be quite similar. At the end of the simulation, we see the following data printed out:

```
Na ionCh: [ 187 128  90 120] mV/s
Na pump: [-160 -158 -157 -158] mV/s
K ionCh: [ -65 -40 -61 -107] mV/s
K pump: [ 107 106 105 105] mV/s
Cl ionCh: [ 68 35 -24 -40] mV/s
```

Totals by ion from all sources:

```
Na total: [ 27 -31 -68 -38] mV/s
K total: [ 41 66 44 -2] mV/s
Cl total: [ 68 35 -24 -40] mV/s
```

Grand valence-weighted totals from all sources across all ions:

```
[ 0 0 0 0] mV/s
```

The topmost set of data is the flux from ion channels and ion pumps for each ion and each cell.

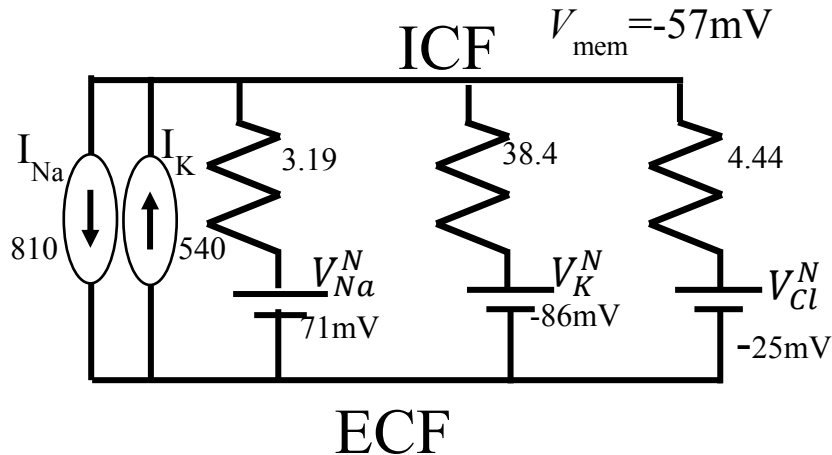
The middle set of data then sums ion channels with pumps. For, e.g., Na in cell #1, we have 128-158= -31 (given some roundoff error). Note that every single cell has a nonzero flux of every single ion, so we are most certainly not at steady state. However, we *are* at quasi steady state. For, e.g., cell #0, we see fluxes of Na and K summing to 27+41=68 mV/s; this exactly balances the Cl flux (since the former are positive and Cl is negative). The same is true for cell #1 (-31+66=35), and for cells #2 and #3 as well. Thus, every cell has zero *net* charge flowing in.

The final set of “grand total” data says exactly that – in each cell, the total amount of charge flowing in is zero. I.e., we are at QSS.

2. In class, we discussed a model of quasi-steady-state  $V_{\text{mem}}$  that worked as a linear system. The debugging function `edb.analyze_equiv_network()` looks at the underlying physics and prints out

the equivalent  $V_N$  and  $G$  for each ion. For run #1, you should see each cell having the same  $V_{Nernst}$  (because they all have the same initial ion concentrations), but different conductances (since we changed the ion-channel diffusion constants). Do the numbers it prints match your observed data for run #1 pretty well? I.e., using circuit analysis, do all of the  $V_N$  and  $G$  values, assembled into an equivalent circuit, correctly result in zero net current into the cell? To minimize busy work, just do this for cell #2.

The equivalent circuit for a cell in QSS is redrawn here (from the slides we covered in class), using the numbers we gave for cell #2:



Let's look at the different currents:

- $I_{\text{pump,Na}} = 810$  moles/( $\text{m}^2 \cdot \text{s}$ ) leaving the cell
- $I_{\text{pump,K}} = 540$  moles/( $\text{m}^2 \cdot \text{s}$ ) entering the cell
- $I_{\text{IC,Na}} = (71\text{mV} - -57\text{mV}) * 3.19$  moles/( $\text{m}^2 \cdot \text{s} \cdot \text{mV}$ ) = 408 moles/( $\text{m}^2 \cdot \text{s}$ ) entering the cell
- $I_{\text{IC,K}} = (-86\text{mV} - -57\text{mV}) * 38.4$  moles/( $\text{m}^2 \cdot \text{s} \cdot \text{mV}$ ) = 1114 moles/( $\text{m}^2 \cdot \text{s}$ ) leaving the cell
- $I_{\text{IC,Cl}} = (-25\text{mV} - -57\text{mV}) * 4.44$  moles/( $\text{m}^2 \cdot \text{s} \cdot \text{mV}$ ) = 142 moles/( $\text{m}^2 \cdot \text{s}$ ) leaving the cell

For the ion channels, we determined whether each ion is entering or leaving the cell as we did in class, by comparing  $V_{\text{mem}}$  to that ion's  $V_{Nernst}$ , and adjusting the result for whether the ion is positive or negative. For, e.g., Na,  $V_{\text{mem}}$  (-57mV) is substantially lower than  $V_{Nernst,Na}$  (71mV); this negative charge in the cell will pull Na in. For Cl,  $V_{\text{mem}}$  (-57mV) is substantially lower than  $V_{Nernst,Cl}$  (-25mV); this negative charge in the cell will push Cl out.

The total charge entering the cell is thus  $(-810 + 540 + 408 - 1114 + 142) = -834$  moles/( $\text{m}^2 \cdot \text{s}$ ). Note that we have reversed the sign on Cl, since it is a negative ion. In principle, at QSS this should be zero, and clearly it is not! The reason is that our simple linear model is not perfect; Bitsey actually does not use this model, but uses the more accurate Goldman-Hodgkins-Katz (GHK) model.

Viewed slightly differently, it would have taken a  $V_{\text{mem}}$  of roughly -75mV to create QSS if one believed the numbers that our linear model gave us. That's perhaps not too far off from -57, but not that great either.

3. What were your results for the modified run? Hopefully, you found that the system is quite resilient to small changes in initial concentrations. Can you explain why?

As the graph above shows, while all four cells started at different initial  $V_{\text{mem}}$  in lab2b, they all wound up with identical  $V_{\text{mem}}$ . This is to be expected. As we discussed in class, the cell acts as a negative-feedback machine to reach QSS. The ion concentrations (which specify the Nernst voltages), together with the ion-channel conductances, work together to create a desired  $V_{\text{mem}}$ . If the current  $V_{\text{mem}}$  is less than that, then net positive charge will flow into the cell until we reach that  $V_{\text{mem}}$ ; on the other hand, if the current  $V_{\text{mem}}$  is too high, then net negative charge will flow into the cell until we reach that  $V_{\text{mem}}$ . In either case, the negative-feedback system will keep working until we reach the desired  $V_{\text{mem}}$ . As slide #36 of the QSS slide deck notes, the final  $V_{\text{mem}}$  in QSS depends on the ion-channel conductances, but it does not really depend on ion concentrations. Note finally that in a very detailed sense, ion concentrations do affect  $V_{\text{Nernst}}$ , and  $V_{\text{Nernst}}$  does affect  $V_{\text{mem}}$  – but the small initial-concentration changes that we made are far too small to have any noticeable affect on  $V_{\text{Nernst}}$ .

You should turn in two files: your main.py and a report with the graphs and the answers to the questions.