Practical 2: The Hodgkin-Huxley model

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1 Aims

This practical has three aims:

- To investigate properties of the action potential (**AP**), as observed under current clamp conditions
- To use voltage clamp and ion-selective blocking to investigate ionic currents
- To show how the Hodgin-Huxley (**HH**) model accounts for these properties

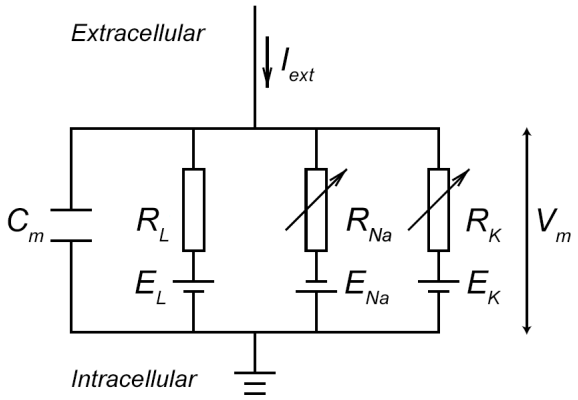

Figure 1: Schematic diagram of the equivalent circuit for the HH model (adapted from *Gerstner, W., Kistler, W., Naud, R., & Paninski, L. (2014). Neuronal Dynamics: From Single Neurons to Networks and Models of Cognition. Cambridge: Cambridge University Press. doi:10.1017/CBO9781107447615*). The injected current with a direction towards the intracellular compartment is, by convention, positive. Note the direction of the batteries for the different channels. E_L (leakage reversal potential) and E_K (potassium reversal potential) are both negative, which means that current through these channels would tend flow *out* of the cell (only when the membrane potential is more positive than the reversal potentials, which is normally the case). In standard circuit notation, current flows out of the positive pole of the battery (long line). In contrast, E_{Na} (sodium reversal potential) is quite positive, and it is thus depicted accordingly. The arrows crossing R_{Na} and R_K signify that these are variable. Can you explain why?

2 Constructing the HH model in NEURON

In Figure 1 above you can see the equivalent circuit for the famous model that Hodgin and Huxley built in their seminal studies carried out in the squid giant axon (Hodgkin AL, Huxley AF. The Journal of Physiology, 1952d). For this work they later quite deservedly won the Nobel Prize in Physiology or Medicine in 1963.

Let's follow their steps and construct a NEURON simulation of this model. First, type:

create soma
access soma
soma insert hh

(Remember that in the first practical we inserted only passive leakage channels with *soma insert pas* instead. Now we insert HH-type channels) You can always use *psection()* to view the different parameters and their values. In this case, the values correspond to the ones used by Hodgkin and Huxley in their original publications, such that $g_{\bar{Na}} = 0.12 S/cm^2$, $g_{\bar{K}} = 0.036 S/cm^2$, and $g_{\bar{L}} = 0.0003 S/cm^2$.

Now, bring up a **RunControl** window and a **Voltage graph** as described in Practical 1. Run the simulation for $100ms$. There are no input currents to the neuron at this point. Select V**iew**$\longrightarrow$**View=Plot** to see the trace in detail. You should be able to see something like Figure 2. What is the value of resting membrane potential in this case? Why do you think the membrane potential behaves this way? Meaning that, since there is no external current being applied, what is causing the change in the membrane potential? How can you find out the cause?

(Hint: you have to look at the currents of the model. According to the HH model, the total current flowing in the circuit is given by $I_{total} = I_C = I_{Na} + I_K + I_L + I_{ext}$, with $I_x = g_x(E_x - V_m)$, for each current x)[1]

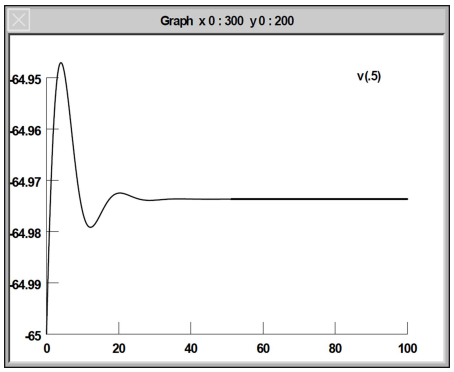

Figure 2: No-input behaviour of membrane potential

3 Current clamp

Now, let's inject some current into the neuron to make things more interesting. You can do this by selecting **Tools**$\longrightarrow$**Point Processes**$\longrightarrow$**Managers**$\longrightarrow$**Electrode** from the **Main menu.**

3.1 Fundamental behaviour

In the **I/V Clamp Electrode** window that shows up, select **IClamp.** Then, change the **delay** to $10ms$, the **duration** to $1ms$, the **amplitude** to $2nA$, and **tstop** to $40ms$. Also, select the **Keep lines** option in the **Voltage graph**. Now run the simulation 8 times, increasing the **amplitude** by $2nA$ each time. Figure 3 shows what you can expect to see. What can you observe with increasing inputs? Discuss with your neighbour.

[1] So, you could bring up a new plot and with **Plot what?** choose to look at the conductances for the different channels. Do these change? It's best that you do this for one conductance at a time to be able to see the trace with sufficient detail. Look at the dynamics of these changes and compare them to the membrane potential dynamics.

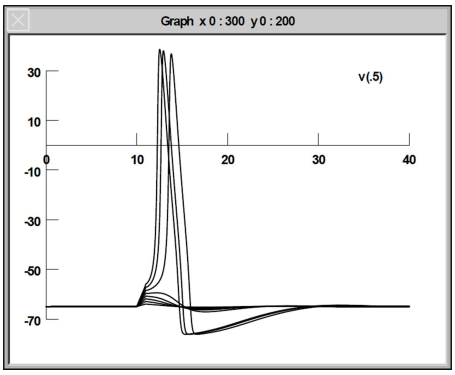

Figure 3: Membrane response with progressively larger input currents

3.2 The spike threshold

Technically, there is **no** absolute *current* threshold for spike generation in the HH model, meaning that the current needed to elicit an AP is differs depending on the stimulation conditions. To convince yourselves that this is true, first try finding what the lowest **amplitude** that elicits a spike is, without changing any other parameter. Do this with a precision of 4 significant figures.[2]

Now, decrease the stimulus **duration** to $0.5ms$. Make a prediction for the range of the required current. Try out your predictions and find how close you were (it would be best if you delete the previous traces so that it does not get too crowded). Next, try the opposite, increase the **duration** to $8ms$ and identify the minimum current in that case. Hopefully, by now you have realized that the notion of a *current* threshold does not make much sense in this context.[3]

However, a *voltage* threshold can be helpful and is indeed used commonly in integrate-and-fire neuron models. In your previous simulations, you should be able to identify an approximate value of the membrane potential that, when reached, is followed by an AP. Is there such a sharp voltage threshold across all stimulation conditions?

3.3 Refractoriness

Neurons have a refractory period, a period of time after initiation of a spike that another AP cannot be elicited. There are two phases of the refractory period, the *absolute* refractory period, during which no matter the amplitude of the current injected, no AP can be initiated, and the *relative* refractory period, during which APs are inhibited, but not impossible to elicit.

To study this behaviour of our model, we need a second electrode. You can do this following exactly the same steps as above.

Let your first electrode have **delay, duration,** and **amplitude** values of 10, 1, and 14, respectively. For the second electrode, let **duration** be $1ms$ as well, and set the **amplitude** to the minimum value you found in the beginning of section 3.2. Then, by changing the delay of the second electrode pulse, measure the *relative* refractory period of our cell (at most 2 significant figures).

[2]The best way to home in on a value would be to split the difference between an affirmative and a negative answer. For example, if a value of 3.5 does not elicit a spike and 4 does, then you should try 3.75. If this elicits a spike, then you try 3.875 etc..

[3]Intuitively, this makes sense, because what matters eventually is how much **charge**, Q, has moved across the membrane. Remember that $I = \Delta Q/\Delta t \Longrightarrow \Delta Q = I * \Delta t$, which means that if we halve the pulse time, but double the amplitude, the charge transferred will be the same. The only difference will be how quickly the charge is transferred, and thus how quickly the membrane potential changes. In the limit, where the current pulse gets infinitestimately short (and the current is large enough to keep ΔQ constant), there would be a jump in the membrane potential to a new value. You could see this for yourselves in the simulations by playing around with the parameters.

Now, try to find the *absolute* refractory period of the cell.[4] Beware, not everything that looks like an AP is one. To check if it is, you should be able to observe a nonlinear change in the behaviour of the membrane potential at some value of injected current. If the response grows linearly with the injected current, then it's not an AP!

Try to remember (or better yet, reason) what mechanisms are responsible for the existence of the refractory period. Are the two phases of the refractory period caused by the same mechanism(s)? Do you know of any neurotransmitters or drugs that take advantage of these mechanisms? Discuss your ideas with your neighbour.

At the end of this section, you can close one of the electrodes by pressing its **Close** button.

4 Repetitive firing and the F-I curve

Until now, we have only injected currents long enough to elicit a single AP. However, with longer current pulses multiple APs can be generated. To explore this behaviour, set the following parameters for the current pulse:

delay: $20ms$
duration: $100ms$
amplitude: $10nA$

If you now run the simulation again for $200ms$, you should see a train of spikes like in Figure 4, below.

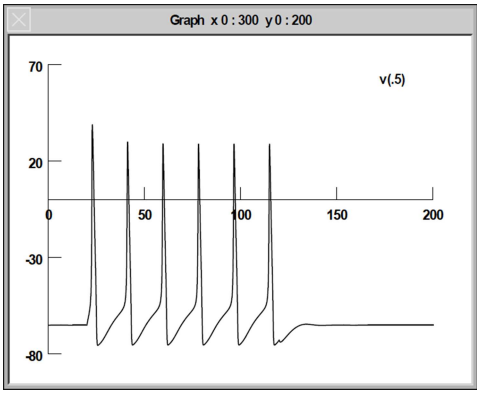

Figure 4: A train of APs generated by a long current pulse

Now, systematically change the current **amplitude** to explore its relationship with the resulting spike frequency.[5] It would be best to note down the current values you use and the resulting AP frequencies.

Once you think you have enough data points collected, let's try to plot the *F-I curve* (firing rate versus input). We can do this in MATLAB (also installed on DICE computers), as a warmup for our next practicals. You can put your data in two vectors and then use the function *plot* to visualize the curve.[6] The curve will look approximately like the one in Figure 5, but depending on the number of data points you choose to collect it might look more or

[4] Hint: To do this you have to change both **delay** and **amplitude** of the second electrode

[5] To get the frequency of spikes in Hertz (Hz), you have to divide the number of spikes occuring in a period of time, by the duration of that period (in seconds).

[6] For those not familiar with MATLAB programming, this is a good chance to take your first steps. In MATLAB, we usually work with matrices (that's where it gets its name). *Vectors* are one-dimensional matrices and come in two types, *row*-vectors (horizontal 1D matrices) and *column*-vectors (vertical 1D matrices). For our purposes, it doesn't matter which ones we use (in fact, we could just as easily use a 2D matrix, but that would be a bit more complicated), so we'll use row-vectors.

To create a vector, you have to assign it to a *variable* name (this is how it is stored in and retrieved from memory). So, you could have a vector for amplitudes, as *currents = [1, 2, 3, 4, 5, 10, 15, 20]* (or whatever values you choose to use) and another for the resulting AP frequencies (or AP number, the shape of the curve will be identical either way), as *freq = [0, 0, 0, 10, 10, 60, 70, 80]* (or whatever values you end up with from the simulation).

Once you have created those variables you can type in *plot(currents, freq)*, to look at the resulting F-I curve (*plot(x, y)* is a built in *function* in MATLAB that takes two variables, x and y, and plots them in a 2D plot against each other, in the respective axes) You can make your figure nicer by including labels and units for your axes. You can do this by using the functions *xlabel('*typeinthelabelyouwant*')* and *ylabel('*typeinthelabelyouwant*')* for the axes labels and *title('*typeinthetitleyouwant*')* for the figure title that appears at the top. If

less detailed. Don't spend *all* your time trying different amplitudes, but you should have enough points to be able to have a sense of the behaviour.

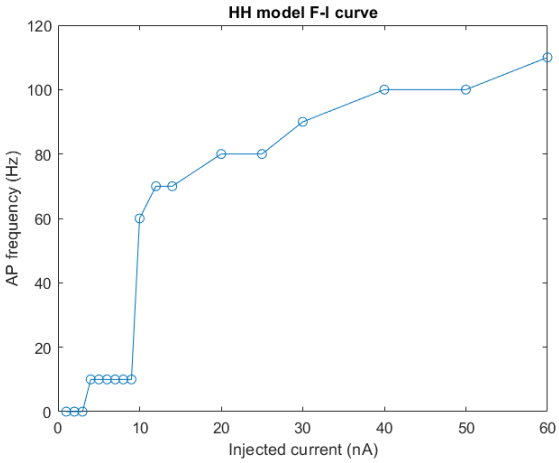

Figure 5: The F-I curve in the HH model

4.1 Blocking ionic currents

During their studies, Hodgkin and Huxley, had to systematically change the concentration of Na^+ and K^+ to study their effects on the action potential. Since that time, however, pharmacological blockers of ion channels have been discovered and developed.

Tetrodotoxin (TTX) is a Na^+-channel blocker found in the pufferfish, Fugu, in Japan. This is what makes eating the fish potentially lethal, if prepared by non-specialists. The toxin blocks AP generation and conduction, and thus leads to paralysis and asphyxiation of the victim. Tetraethylammonium (TEA) is a voltage-gated K^+-channel blocker (but has effects on other channels as well).

We can simulate the effect of these drugs by changing the values of the ionic conductances in our model. We could do this from the command line, but another way is to select **Tools**⟶**Distributed mechanisms**⟶**Viewers**⟶**Shape name.** This brings up a dialog box with a list of sections in the bottom right corner. Double-click on **soma**. You should now have a dialog box with a number of parameters in it. For the steps that follow, try to predict what the behaviour of the model will be *before* you apply each manipulation. Then check if the results agree with your predictions.

- To block the K^+ conductance, set **gkbar_hh** to 0 and rerun the simulation. Is this what you expected to see? What does this mean? Return **gkbar_hh** to its original value by clicking on the red ticked box next to it.
- Now swap to blocking the Na^+ conductance by setting **gnabar_hh** to 0. Is this what you expected to see? What does this mean?
- (Optional) Take intermediate values of the densities and take higher than normal as well. Does the behaviour follow your intuition ?
- (Optional) Research how the F-I curve changes with different densities settings. Restore the original settings by clicking on the red ticked boxes before continuing.

you want to go fancier, there are options about line width, color, style etc. Just type *plot* at the search bar on the top right corner of the main window to look at the documentation for the function. You can search for information about any MATLAB function in this way.

5 Voltage Clamp

Convert the current clamp to a voltage clamp by clicking on its **VClamp** button. Set the *Testing Level* **duration** to $15ms$ and its **amplitude** to $0mV$. Click on the **VClamp.i** graph button at the bottom of the **I/V Clamp Electrode** window. A current axis should appear. Now run the simulation for 15ms. We will try to indentify the different components of the current.

First, block just the Na^+ current and run the simulation again. Then, unblock the Na^+ current, block the K^+ current and run the simulation again. Discuss your observations with your neighbour. Which of the terms *activation* and *inactivation* apply to the currents you see? Are the currents inwards or outwards? Finally, block both currents. What is left ?[7]

6 Putting it all together

We now study the individual currents during a spike. Go back to current clamp, and make the neuron spike a single AP as before. Make sure that all currents are unblocked again.

Open a **State axis** from the **Graph** menu and plot **soma.m_hh**, **soma.h_hh** and **soma.n_hh** in this graph. Open a **Current axis** and plot the conductances **soma.gna_hh, soma.gk_hh**, and **soma.gl_hh**.

It should look something like the following:

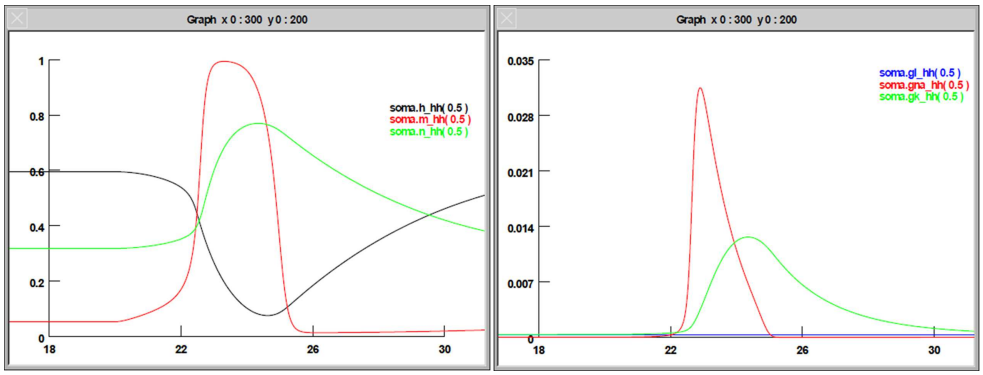

Figure 6: Gating variables (left) and ionic conductances (right) in the HH model during an AP.

Which of the gating variables m, h, and n are activating/inactivating? Explain how the two graphs are related to each other.

7 (Extra) Spikes without K channels

(In case you thought you understood it). It turns out to be possible to generate spike-like events without a K^+ conductance. Block the K^+ conductance and play with the other conductances (including leak conductance) to show this effect. What is the advantage of the biological solution of having K^+ channels?

8 (Extra) Temperature

The temperature has a strong effect on the HH equations. Let's warm the squid to room temperature (*celsius = 20* on the command prompt). Look at the spike frequency and the shape and amplitude of the spikes at this temperature. Research the channel densities required at higher temperatures.

[7] You could also look at all of the above with a normal **Current axis** window, in which you would choose to plot the relevant currents from the **Plot what?** selection window. In this way you can have multiple currents shown at the same time.

9 Checking your conceptual understanding

After finishing this lab session you should be able to answer the following questions (some of them you might have already seen in the main body of these instructions):

1. Which ion is responsible for the repolarization of the cell membrane after an action potential is fired?
   (a) Na^+
   (b) Ca^{2+}
   (c) K^+
   (d) Cl^-
2. The relative refractory period of a neuron is caused by:
   (a) Inactivation of voltage-gated Na^+ channels
   (b) Closing of leakage ion channels
   (c) Opening of voltage-gated Ca^{2+} channels
   (d) Delayed closing of voltage-gated K^+channels
   (e) The action of the $Na^+ - K^+$ ATPase
3. Which parameter in the HH model reflects the inactivation of Na^+ channels?
   (a) m
   (b) h
   (c) n
   (d) All of the above
4. The inactivation of Na^+ channels is caused by:
   (a) Blockage by Mg^{2+} ions
   (b) Channel endocytosis
   (c) A conformational change in the protein that blocks the channel pore
   (d) All of the above, depending on the neuron type
5. The rising phase of the action potential is controlled by the parameter:
   (a) m
   (b) h
   (c) n
   (d) All of the above
6. Which one of the following is the fastest process:
   (a) Opening of K^+ channels
   (b) Closing of Na^+ channels
   (c) Inactivation of Na^+ channels