The Action Potential

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1 Introduction

In this tutorial, we consider the probably most famous and influential model in neuroscience: the Hodgkin-Huxley model. This quantitative model, which was directly based on empirical results from experiments on the squid giant axon, describes the dynamics of the different ion channel types underlying action potential generation. If you understand the concepts covered in this tutorial, you have a very good understanding of cellular electrophysiology. Enjoy!

2 Ion Channels and Conductances

You might recall that the current flowing through a simple resistor is proportional to the voltage across it. We denote the voltage with the letter *V*, the current with *I*, and the resistance with *R*. Ohm's law tells us how current ("input") and voltage ("output") relates to each other:

$$V = R\,I$$

Electrophysiologists prefer to talk about conductance *G* which is the reciprocal value of resistance:

$$G = 1 / R$$

And therefore

$$I = G\,V.$$

Ohm's law can be applied to resistors (electric circuit components), biological cell membranes, and many other physical entities. The concepts of "resistance" and "conductance" are abstractions or models of complicated physics that we can ignore. Here, conductance is a property of the cell membrane. Ions (the charge carriers in biological systems) cannot pass through a cell membrane, except if there are pores called ion channels. The number of channels per given area of membrane and the permeability of each channel defines how well ions can pass through a patch of cell membrane. Therefore, we can model the total of all channels as a conductance. The more (open) channels there are, the higher the conductance (and therefore lower the resistance) is. Such membrane conductances have three main properties:

1. We consider here channels which are permeable to just one given ion type. We introduce a conductance for each channel type since they have different characteristics. Here, we consider channels selective to potassium and sodium ions. The corresponding conductances are denoted by G_K and G_{Na}, respectively.

2. Both the potassium and the sodium channels are voltage-gated. This means that their permeability depends on the membrane voltage. In other words, both conductances, $G_K(V)$ and $G_{Na}(V)$, are a function of the membrane voltage V.

3. When the membrane voltage changes (e.g. when a constant depolarizing current is injected), it takes some time until the conductances reach a new steady-state value. We denote this time-dependence as $G_K(V, t)$ and $G_{Na}(V, t)$, respectively.

With the above, we now have a grip on our initial aim of describing the ion channel dynamics. All we need to know is $G_K(V, t)$ and $G_{Na}(V, t)$. The issue is that these conductance time-courses are the solution of differential equations. Remember that differential equations have functions as solutions, and can be solved numerically using a software package such as SIMULINK (part of Matlab). You will learn in this tutorial how to do this.

3 Voltage-Gated Conductances

In this section, you will learn how Hodgkin and Huxley modeled such time- and voltage-dependent ion channel conductances. Starting point is that a single, voltage-gated channel fluctuates between the open and closed state in a probabilistic manner (like a coin). The probability of being in the open state is a function of the membrane voltage. However, due to the high-density of such channels, we here do not care about individual channels but focus on a macroscopic description. Two type of ion channels are considered here: channels which open and stay open when the cell is depolarized (*persistent conductances*) and channels which open and then close again when the cell is still depolarized (*transient conductances*).

3.1 Persistent Conductances

The first class of channels considered here are channels that have a gate that opens (*activation*, ions can flow) and closes (*deactivation*, ions cannot flow). Note that this is just a simplifying model, in reality a number of conformational changes need to occur such that a channel is open. In the case of the potassium channel considered here (also known as delayed rectifier, for reasons you will see soon), four independent and identical subunits need to undergo such a structural change. If we call the probability that a given subunit is in its open state n (and correspondingly $1 - n$ the probability that a subunit is its closed state), the probability p that the channel is open is given by:

$$p = n\,n\,n\,n = n^4$$

The variable *n* is also called gating variable and ranges between 0 and 1. The dynamics of a single subunit is given by

$$dn/dt = \alpha_n(V)(1 - n) - \beta_n(V)n.$$

As already mentioned earlier, this is a differential equation since both *n* and its derivative *dn/dt* occur in the equation. The transition rates α_n (V) and β_n (V) describe the probability of activating or deactivating an individual subunit. Remember that such a differential equation expresses how the rate of change, *dn/dt,* depends on *n*. Here, only channels that are closed (with probability 1-n) can get open (with probability α_n (V)), the full term is the product of the two probabilities. Similarly, only channels that are open (probability n) can close (with probability $\beta_n(V)$) the minus sign in the above equation makes sense when you consider that the channels that close reduce *n.* These functions were chosen to fit Hodgkin and Huxley's experimental data. After algebraic manipulation, this differential equation can be rewritten in the following form:

$$\tau_n(V)\, dn/dt = -n + n_\infty(V)$$

Both the steady-state value $n_\infty(V)$ and the time constant $\tau_n(V)$ are a function of the membrane voltage (as shown in Figure 1, blue traces). We see from these plots that depolarizing the cell leads to an increased activation of the voltage-gated potassium channels.

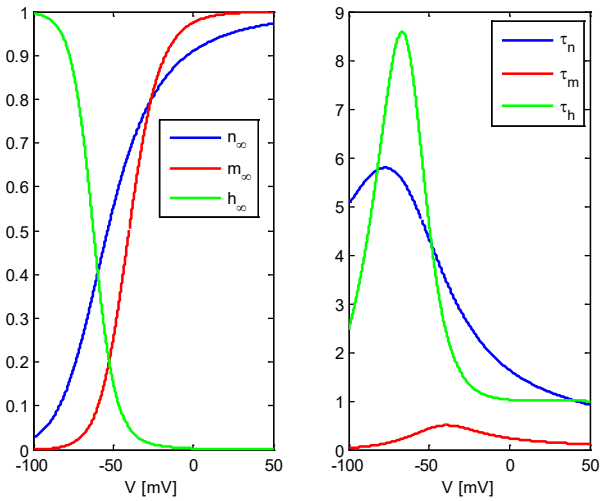

Figure 1: Steady state values (left) and time constants (right) for potassium activation *n*, sodium activation *m*, and sodium inactivation *h* as a function of membrane voltage *V*.

Putting everything together, the total potassium conductance G_K is given by the product of the maximal conductance g_K (all channels open) and the probability of a given channel to be in the open state:

$$G_K = g_K n^4.$$

3.2 Transient Conductances

Transient conductances are used to describe channels which open only transiently upon depolarization since there are two mechanisms working in opposite directions. Therefore, we have now two instead of one variable describing the opening and closing of these channels. For the transient sodium current which we consider here, there is activation *m* (same concept as activation *n* in case of the potassium current) and inactivation *h* describing the process working in the opposite direction. In order for such a channel to be permeable to sodium ions, the three activation gates must be in their open state and the inactivation must be open, as well (deinactivated):

$$p = m^3 h$$

where both *m* and *h* are given by differential equation of the form as for *n* (you are spared from the details) but with different formulae for the transition rates α_m, α_h, β_m, and β_h. As in the case of the potassium current, the steady-state values for both activation *m* and inactivation *h* are shown in Figure 1. From this plot, we see that depolarizing the cell leads to an increase in activation *m* (opening of channels) and a decrease in *h* (inactivation of channels). Inactivation *h* has a slower time-course than activation *m*. Putting everything together, the total sodium conductance G_{Na} is given by the product of the maximal conductance g_{Na} and the probability *p* of a given channel to be in the open state:

$$G_{Na} = g_{Na} m^3 h.$$

3.3 Ionic currents

Congratulations! If you reached this point, you worked yourself through the hardest part of this tutorial. From now on, things are just getting easier. First, we want to describe the currents carried by the different ion types through the ion channels which we characterized above. Again, we follow Ohm's law, however we need to take into account the reversal potentials of the different ion types. Remember that the reversal potential denotes the membrane voltage at which there is no net flux of a given ion type across the cell membrane (balance of drift and diffusion) and is modeled as a battery (voltage source).

The potassium current is given by:

$$I_K = g_K n^4 (V - E_K)$$

Similarly, we find for the sodium current:

$$I_{Na} = g_{Na} m^3 h (V - E_{Na}).$$

Let's assume you can dissociate these two currents by pharmacological means. In other words, you have your cell and the only current which flows is one of the two currents above. As stated at the end of the last section, we want to quantify the conductances both in their voltage- and time-dependency. How would you dissociate the effect of time and the effect of voltage? The

answer was developed by Cole and applied by Hodgkin and Huxley and is called voltage-clamp. Voltage clamp is an experimental set-up where the membrane voltage is help constant by an electronic circuit such that we can isolate the time-dependent behavior of the conductances for a given so-called holding potential. We will now do exactly the same thing in simulation.

Negative or Positive? Inward or Outward?

Direction and sign of ionic currents are usually a source of confusion. The direction of a current is defined by the direction of flow of positive charge carriers. Here, currents flowing outwards (from cytosol to extracellular space) are of positive sign. Therefore the potassium current will be positive (under normal conditions) since positively charged potassium ions flow out of the cell. In contrast, the sodium current is negative since positively charged sodium ions flow into the cell (under normal conditions). Just to add extra confusion, an injected, external current is positive when it depolarizes the cell (opposite direction / sign from the ion currents). That is why you will see a minus sign sneaking into the equations where we add currents.

4 Exercise A

In this exercise, we will investigate the above introduced persistent potassium current.

1. Open the file `IPotassium.mdl`.

> `IPotassium.mdl`
> This model is an implementation of the persistent potassium current for a specified holding potential. In the block labeled `Membrane Voltage`, the holding potential can be specified by double clicking. The subsystem in the middle computes the potassium current, you can doubl eclick it to see the implementation. The block on the right (`scope`) displays the time-course of the potassium current (once you run the simulation).

2. Make sure the holding potential is set to -30 mV. Run the simulation by clicking the `play` button or `Simulation...Start`. Look at the resulting time-course by double-clicking the scope. Make a sketch of the response in the coordinate system given in Figure 2. Do you see why this is a persistent current?

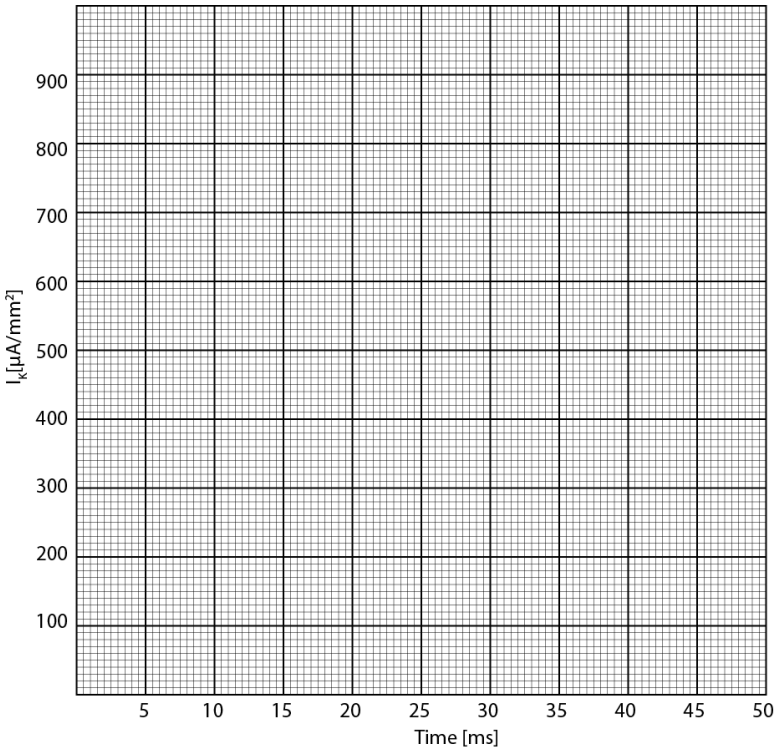

Figure 2: Coordinate system for the time-course of the potassium current. Membrane voltage help at V = -30 mV.

3. We will now determine an I-V-plot for this current. Such a plot shows the current I-V-plot as a function of the voltage (holding potential). In the previous step, we have seen that the current is a function of time for a constant membrane voltage. Here we want a single value for each value of the holding potential such that we have pairs (V, I) which we can use to draw the I-V-plot. A common approach is to take the peak value of the current. Hence, run the simulation for each value of the holding potential given in the table below, and read out the peak values (here this corresponds to the steady-state value for t = 50 ms). In order to read out the peak values, it might be of help to use the zoom button (”magnifying glass”) in the scope window. Fill in your values here:

V [mV]	-80	-60	-50	-30	-20	-10
$I_K[\mu A/mm^2]$						
V [mV]	-10	0	10	20	30	40
$I_K[\mu A/mm^2]$						

Table 1 Peak value of potassium current as a function of membrane voltage V.

4. Using these values, you can now plot the I-V plot using the coordinate system given in Figure 3. Can you see why this current is called rectifying current (compare the current values for negative and for positive values of V)?

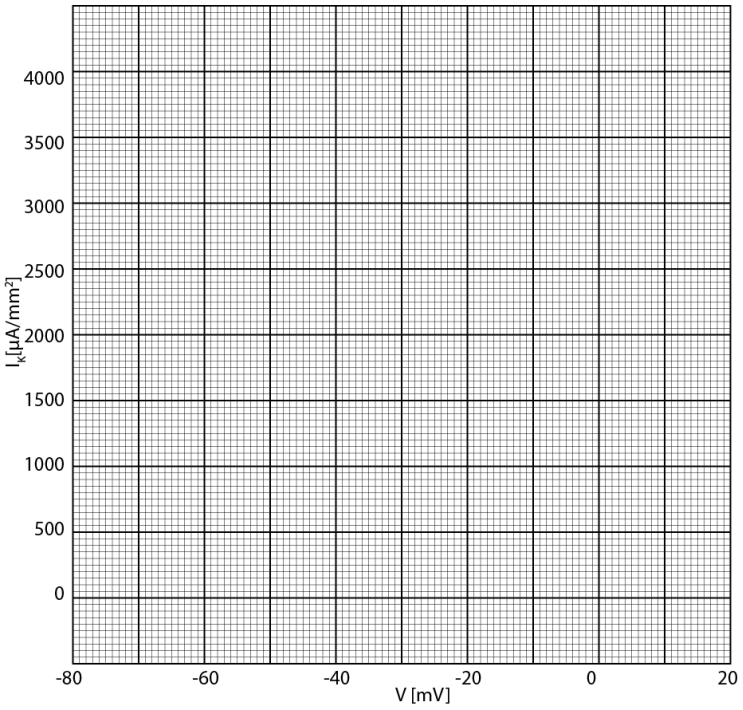

Figure 3: Coordinate system for potassium current I-V-plot.

4. Change the reversal potential of the potassium current by double-clicking the block in the middle and then double-clicking the block labeled ”EK”. Does the I-V relation change as you would expect?

5. Bonus**: Derive the expression for n_∞ and τ_n (as functions of α_n and β_n).

5 Exercise B

We will now do the same thing for the transient sodium current. The procedure is exactly the same as in Exercise A.

1. Open the file `ISodium.mdl`.

> `ISodium.mdl`
> This model is an implementation of the transient sodium current for a specified holding potential (Equation 11). In the block labeled Membrane Voltage, the holding potential can be specified by doubleclicking. The subsystem in the middle computes the sodium current, you can doubleclick it to see the implementation. The block on the right (scope) displays the time-course of the sodium current (once you run the simulation).

2. Make sure that the holding potential is set to -30 mV. Run the simulation and sketch the time course of the sodium current using the coordinate system given in Figure 4.

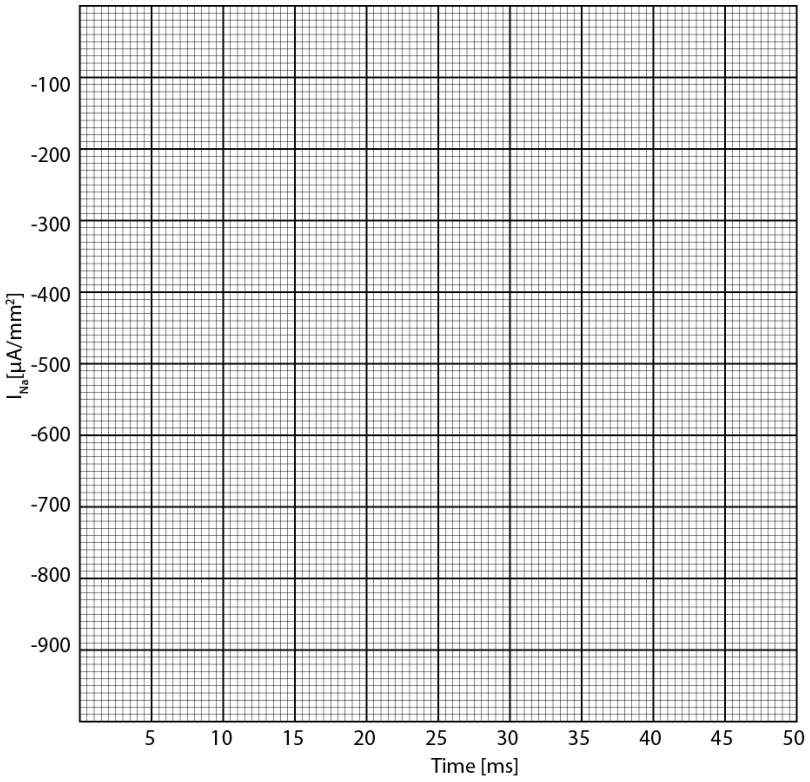

Figure 4: Coordinate system for the time course of the sodium current. Membrane voltage held at V = –30mV.

3. Step through the holding potential (see Table 2 for values) to determine the IV- plot for the peak of the transient sodium current. Use the table below to fill in the values you found. Make sure you read out the peak/trough values and not the steady-state values!

V [mV]	-80	-50	-30	-10	-0	10
I_{Na}[µA/mm²]						
V [mV]	20	30	40	50	60	70
I_{Na}[µA/mm²]						

Table 2 Peak value of sodium current as a function of membrane voltage V.

4. Create the I-V-plot using the coordinate system given in Figure 5.

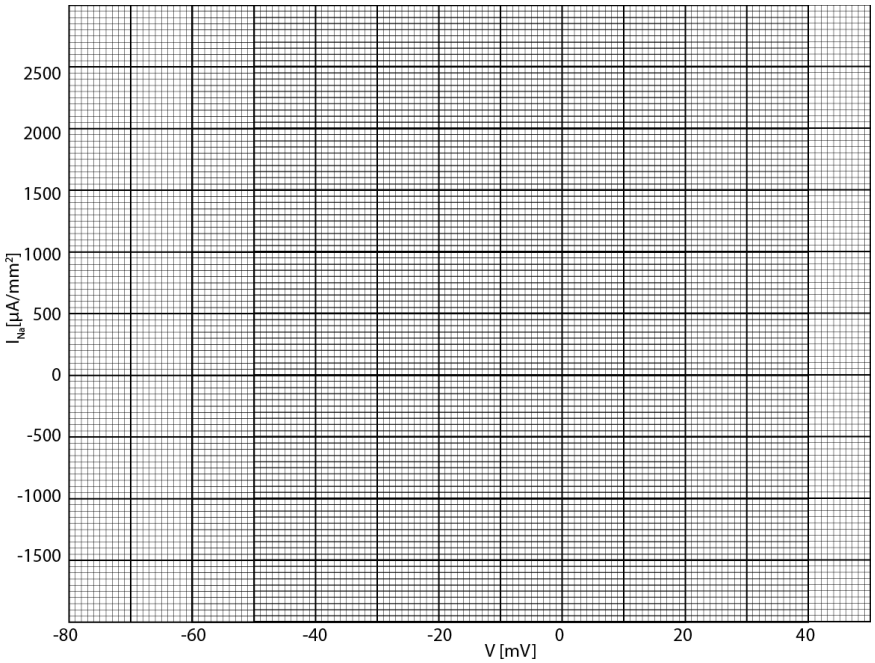

Figure 5: Coordinate system for sodium current I-V-plot.

5. Why does the sodium current not increase monotonically for increased depolarization (as one might expect from the steady-state value of activation *m*, as shown in Figure 1)? Discuss the zero crossing of the I-V-curve.

6. You might have observed that for certain holding potentials there is a non-zero steady-state sodium current. Explain why.

6 Exercise C

Based on the simulations in Exercises A and B, you now know how the peak amplitude of the two current types involved in action potential generation depend on membrane voltage V . But remember, these currents are a function of time for a given membrane voltage. In this exercise we reconsider these currents, but now we are interested in the time it takes until each of these currents reaches its peak value.

1. Go back to the two models `IPotassium.mdl` and `ISodium.mdl`, and compare the time it takes for the potassium current to reach its steady state value to the time it takes the sodium current to reach its peak value (minimum for holding potentials below reversal potential for sodium).

2. How would you characterize the main difference between the two currents? Do you understand why the potassium current is called delayed potassium current?

7 Action Potential Generation

Congratulations, you have reached the final step. You have now enough understanding to explain action potential generation based on the above simulations. Action potentials are transient deflection of the membrane voltage mediated by the two above studied currents. We have seen that sodium current increases in amplitude for increased membrane voltage. So if a significant number of sodium channels are opened (e.g. by a brief depolarizing current pulse), sodium ions flow into the cell, depolarize it and thereby open even more sodium channels. The sodium mediated current increases rapidly by this positive feedback mechanism. However, we have seen that the sodium mediated current is transient and vanishes rapidly (due to inactivation h). Furthermore, with some delay the persistent potassium current is activated, which drives the cell back to its negative resting potential (since potassium ions flow out of the cell). This is a negative feedback cycle since decreasing the membrane potential by outflowing potassium ions leads to a further decrease in the potassium current. As we have seen, the potassium current is slower than the sodium current. Thus, the potassium current will still be active when the sodium current is already shut off. This explains the so-called after-hyperpolarization. Now it is time to have a look at the complete Hodgkin-Huxley model. Note that when we are summing the currents, there are three more currents we need to include, a capacitive current (the cell membrane acts as a capacitor), a leak current, and an externally injected current. Do you see the terms corresponding to these currents in this equation?

$$C\,dV/dt = -\,g_{Na}m^3h(V - E_{Na}) - g_K n^4(V - E_K) - G_L\,(V - E_L) + I_{ext}$$

8. Exercise D

In this exercise, we have a look at the complete Hodgkin-Huxley model. We will investigate how long and how strong a depolarizing current pulse needs to be to elicit an action potential.

1. Open the file `HodgkinHuxleyPulse.mdl`.

> `HodgkinHuxleyPulse.mdl`
> This is the implementation of the Hodgkin-Huxley model as discussed above. The externally injected current is chosen to be a brief pulse. The three blocks to the left are subsystems implementing the transient sodium current, the persistent potassium current, and the leak current. These currents are summed (together with the injected current I_{ext}) and integrated. The block on the right (`scope`) displays the time-course of the membrane voltage current (once you run the simulation).

2 Change both duration ΔT and amplitude I of the pulse by double-clicking the block `Inject Pulse`. Find pairs (ΔT I) which correspond to pulses just enough to elicit an action potential. List the values in the table below (first two rows).

ΔT [ms]							
I [µA/mm^2]							
Q [nC/mm^2]							

Table 3: Current pulses sufficient to elicit an action potential.

You should be able to observe the all-or-none nature of the action potential. Either there is just a small deflection of the membrane voltage (few millivolts) or a full-blown action potential. Note that total charge of a current pulse is given by the product of amplitude and duration:

$$Q = I\,T$$

Does your data support the hypothesis that there is a minimal value of charge for action potential generation? Tip: In order to answer this question, multiply duration and amplitude of your values (last row in above table). What else could play a role whether an action potential is elicited?

3 Have a look at the potassium and sodium current by double-clicking the scope labeled `Ion Currents`. Make sure you understand which trace corresponds to which current and why they are of opposite sign. How do these currents relate the membrane voltage (action potential)?

9. Exercise E

In this exercise, we explore the case where we inject a constant depolarizing current.

1 Open the model `HodgkinHuxleyStep.mdl`.

> `HodgkinHuxleyStep.mdl`
> This is the same implementation of a Hodgkin-Huxley model as in Exercise D. However, the injected current is now a step function and not a pulse anymore.

2 Change the value of the current by double-clicking the block labeled `Inject Step`. The parameter to change is labeled `Final Value`. Run the simulation.

3 Find the threshold for which oscillatory behavior occurs (repetitive firing), for lower values you will find that only a single action potential is elicited.

4 Explore spiking frequency as a function of the injected current. The scope window shows 100 ms, so you can count the spikes and compute the spiking frequency. Once you found the threshold value you can further increase the injected current to determine the so-called f-I-curve which shows spiking frequency as a f-I-curve function of the injected current. List the (f, I) pairs in the table below.

I [$\mu A/mm^2$]							
f [Hz]							

Table 4: Constant bias current I and corresponding spiking frequency f.

5 Draw the f-I-curve. Use the coordinate system in Figure 6.

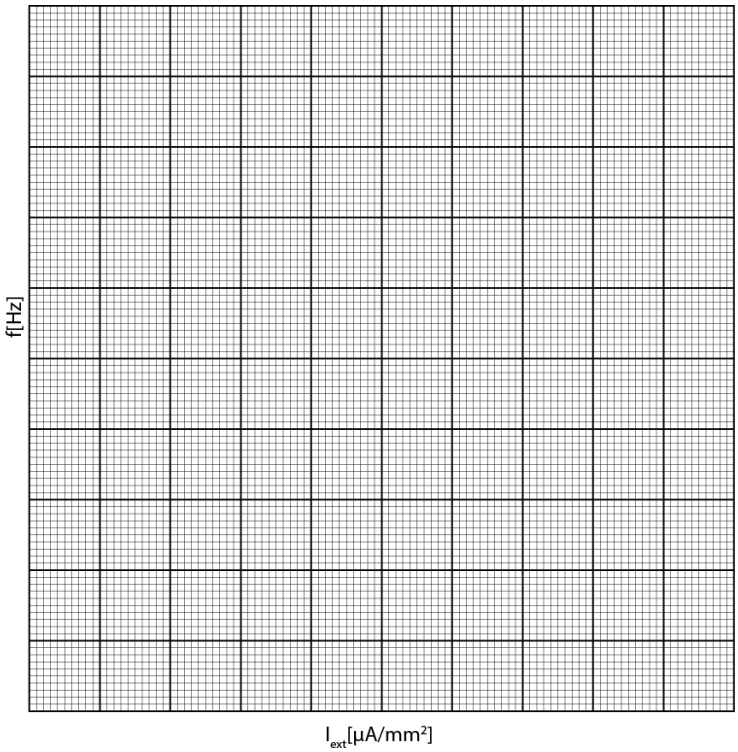

Figure 6: Coordinate system for f-I-plot.

6 Bonus**: Test the response of the model to other input current waveforms (e.g. sine wave or noise) by choosing another source block from the library (SIMULINK...Sources). Refer back to the SIMULINK Tutorial to learn how to change the model for that.