

COMPUTER SIMULATION OF ACTION POTENTIAL GENERATION

The aim of the practical

The purpose of this practical is to investigate *in silico* how the action potential depends on the properties of the voltage-gated sodium and potassium channels. An accurate (quantitative) description of this phenomena requires the language of biophysics, thanks to which we can, not only accurately describe bioelectricity, in particular excitability, but also precisely predict how a change related to a disease, will affect the generation of axon potentials. This practical gives students an idea of how a neurophysiologist describes the basic neurophysiological properties of the nerve cell and the generation of action potentials in daily practice.

Experimental setup

Computer software 'MetaNeuron' for simulating electrophysiological properties of excitable cells, developed by Eric Newman, University of Minnesota.

First part

Aim: The aim of this part of the practical is to familiarize you with the MetaNeuron software and the basic parameters describing the course of the action potential.

Course of the simulations:

1. Open **MetaNeuron** software. From the **Lesson** drop-down menu, select **Lesson 4: Axon action potential**. Based on defined parameters (marked in the figure below), the program calculates the value of membrane potential over time. Notice the red graph at the bottom of the window that shows when the depolarizing stimulus is applied. The duration of the depolarization stimulus is determined by the **Width (ms)** parameter (blue frame in the figure above; the default is 0.1ms). After the threshold potential is reached, an action potential is generated. Software accept only values with dot as a decimal separator.

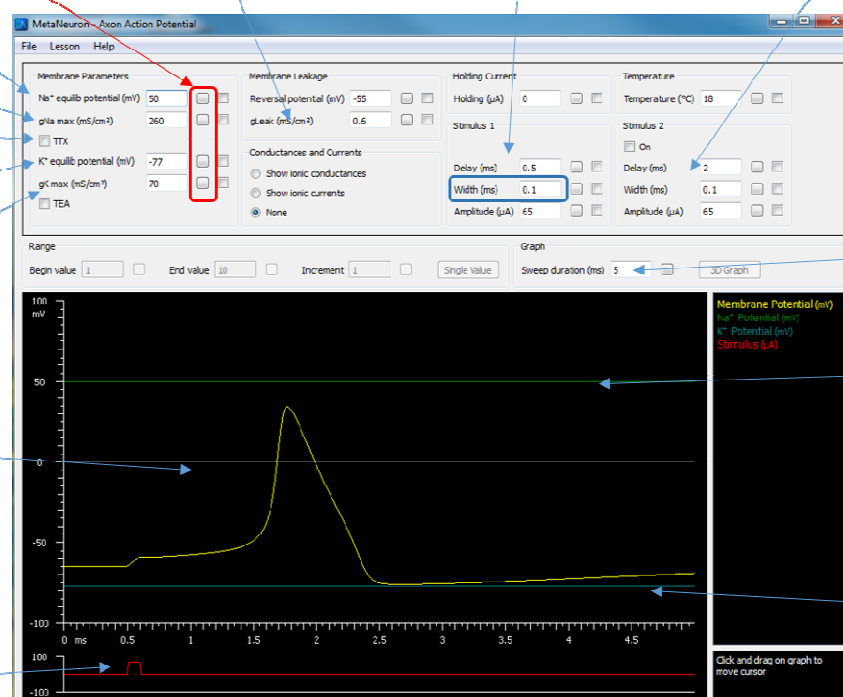
You can change smoothly given parameter by marking these windows with the left mouse button and moving the mouse vertically

Leak conductance

Three parameters that define the first depolarizing stimulus:
Delay - stimulus application time
Width - stimulus duration
Amplitude - the strength of the stimulus

Three parameters that define the second depolarization stimulus. To enable its application, select the On box

Equilibrium potential for sodium ions
Sodium conductance
Tetrodotoxin
Equilibrium potential for potassium ions
Potassium conductance



Duration of simulation

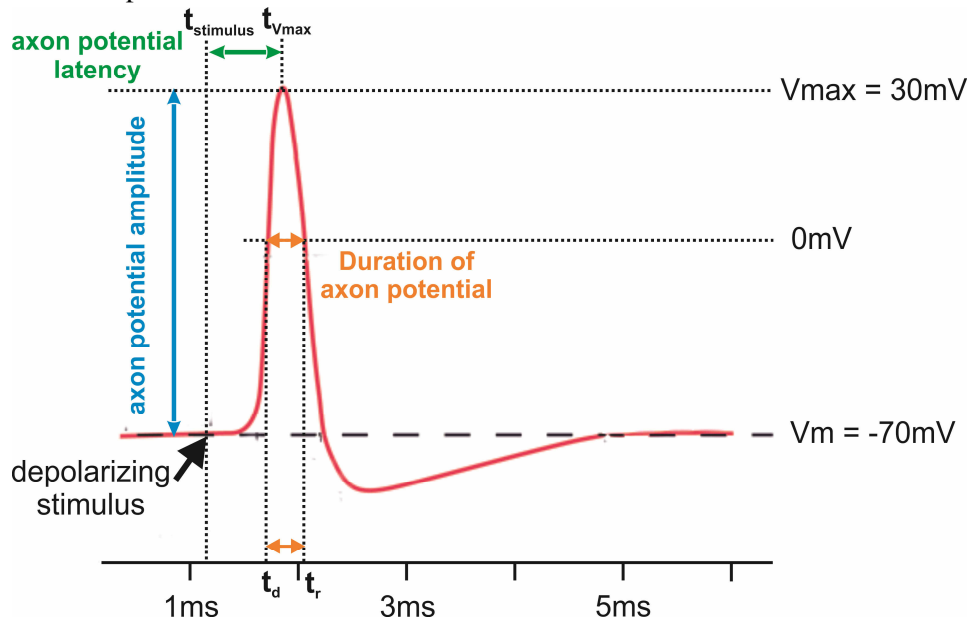
Equilibrium potential for sodium ions

Equilibrium potential for potassium ions

Simulation window. Diagram of changes in membrane voltage (Y axis) over time (X axis)

Scheme of the applied depolarizing stimulus or stimuli

- In obtained form, plot the action potential generated for default simulation parameters. On the graph, mark: (1) resting potential (2), time in which the stimulus was applied, (3) membrane depolarization phase, (4) membrane repolarization phase, (5) hyperpolarization phase.
- Excitable cells often differ in their ability to generate an action potential and its time course. In order to be able to compare the kinetics of action potential and thus, the excitability of the cell, we will introduce three parameters characterizing the action potential: amplitude, duration and latency. The figure below shows each of these parameters.



- The amplitude of action potential** (expressed in mV) is the difference between the maximum value of the membrane potential at the top of the action potential (V_{max}) and the resting membrane potential V_m (amplitude = $V_{\text{max}} - V_m$); in the diagram shown above the amplitude is marked with blue arrow and is equal to: $30\text{mV} - (-70\text{mV}) = 100\text{mV}$.

The duration of action potential can be determined in several ways. For our considerations, we will measure this parameter as the time during which the membrane potential takes a positive value (higher than 0mV). To calculate the duration of the action potential, it is necessary to know the time t_d at which the membrane potential equals 0mV during the depolarization phase and the time t_r at which the membrane potential equals 0mV during the repolarization phase. The duration of an action potential is the difference $t_r - t_d$. In the diagram above it is about 0.3ms (orange arrow).

Action potential latency tells how quickly after the application of stimulus, the excitable cell generates an action potential. In other words, latency (expressed in milliseconds) describes the lag of an action potential relative to the stimulus that triggered it. To calculate the latency of action potential, you need to know the time t_{stimulus} in which the depolarizing stimulus was applied and the time $t_{V_{\text{max}}}$ at which the membrane potential reaches its maximum value. Action potential latency is the difference between $t_{V_{\text{max}}}$ and t_{stimulus} . In the diagram above it is about 0.6ms (green arrow).

- Calculate the amplitude of simulated default action potential, and fill the form. As mentioned, the amplitude of the action potential is the difference between the membrane potential at the top of the action potential and the resting potential. Assuming that the resting membrane potential of the examined neuron V_m is -65mV , the maximum value of the action potential at its peak (V_{max}) should be measured to calculate the amplitude. To do this, while holding the left mouse button, move cursor to the top of the action potential. In the lower right corner of the window, the program will display the value of membrane potential in yellow and indicate the time after the words **Cursor at...**). Then calculate the action potential amplitude from the formula: $V_{\text{max}} - V_m = V_{\text{max}} - (-65\text{mV}) = V_{\text{max}} + 65\text{mV}$.
- Calculate the duration of default action potential. While holding down the left mouse button on the graph, find the times t_d and t_r when the action potential reaches 0mV in the depolarization phase and in the repolarization phase, respectively. Then calculate the duration of the action potential using the formula: $t_r - t_d$. Fill the form.

7. Calculate the latency of default action potential in relation to the depolarizing stimulus; fill the form. The depolarizing stimulus is applied at $t_{\text{stimulus}} = 0.5\text{ms}$ (red scheme below simulation window). Holding the left mouse button down, find the time t_{vmax} at which the action potential reaches its maximum. Then calculate the latency of action potential from the formula: $t_{\text{vmax}} - t_{\text{stimulus}}$.
8. Tetrodotoxin (TTX) is a substance isolated e.g. from the puffer fish. It blocks the activity of voltage-gated sodium channels. Investigate the effect of administration of high concentrations of tetrodotoxin on the course of the action potential. To do this, mark the **TTX** square in the program window. Why is the action potential not generated, fill the form?

Second part

Aim: The aim of this part is to examine the impact of intensity and duration of depolarizing stimulus on the course of action potential.

Course of the simulations:

9. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut).
10. The intensity of depolarizing stimulus is defined by the parameter **Amplitude**. To investigate how the intensity of the depolarizing stimulus affects the course of the action potential, a simulation should be performed when the **Amplitude** parameter takes the values of 50, 100, 150 and 200 μA . For generated simulations, measure the amplitude and latency of action potential as described above. Fill point 2a of the form (mark the lack of an action potential with a dash). Analyze your results and write in one sentence whether the intensity of the depolarizing stimulus affects amplitude and/or latency of action potential (changes of 5% or less of initial value are insignificant).
11. By changing parameter **Amplitude**, find (with an accuracy of 1 μA) the value of the weakest depolarizing stimulus that still generates an action potential (this is the so-called rheobase, fill the form).
12. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut).
13. The duration of the depolarizing stimulus is defined by the **Width (ms)** parameter (blue frame in the first diagram; default value is 0.1ms). In the case of a simulated neuron, the depolarizing stimulus with an amplitude of 10 μA and duration of 0.1 ms is too weak to generate an action potential (check this by running the simulation, set **Amplitude (ms)** to 10, and **Width (ms)** to 0.1). Determine how much you need to extend the duration of the depolarizing stimulus for even a weak stimulus with an amplitude of 10 μA to generate an action potential. When simulating, the **Amplitude (ms)** parameter should be 10 μA , take the **Width (ms)** parameter as 0.1, 0.4, 0.8, 1.2 ms.
14. Find (by changing parameter **Width (ms)**) with an accuracy of 0.1 ms the shortest duration of a stimulus that is already generating an action potential. Complete the table in point 2c of the form. Explain why reaching the threshold potential depends not only on the intensity of the depolarizing stimulus, but also on its duration?

Third part

Aim: In this section, the relative and absolute refractory period of action potential will be examined.

Course of the simulations:

15. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut).
16. We will stimulate the neuron with two stimuli, knowing the time interval between them. To enable the application of the second depolarizing stimulus check the **On** box. To run the simulation: extend the simulation window by changing the **Sweep duration (ms)** value to 10; for the Stimulus 2 set the value 7 in the **Delay (ms)** window parameters (see screen below). The software will stimulate neuron with two identical stimuli (with an amplitude of 65 μA): the first at $t_1 = 0.5\text{ms}$ and the second at $t_2 = 7.5\text{ ms}$, i.e. 7 ms from the beginning of the first stimulus (see red scheme in the lower part of the simulation window).

Thus, the **Delay (ms)** parameter of **Stimulus 2** determines the time after which the neuron was stimulated with the second stimulus in relation to the first.

The screenshot shows the following parameters:

- Membrane Parameters:** Na⁺ equilb potential (mV) 50, gNa max (mS/cm²) 260, TTX, K⁺ equilb potential (mV) -77, gK max (mS/cm²) 70, TEA.
- Membrane Leakage:** Reversal potential (mV) -55, gLeak (mS/cm²) 0.6.
- Holding Current:** Holding (µA) 0.
- Temperature:** Temperature (°C) 18.
- Stimulus 1:** Delay (ms) 0.5, Width (ms) 0.1, Amplitude (µA) 65.
- Stimulus 2:** ☒ On, Delay (ms) 7, Width (ms) 0.1, Amplitude (µA) 65.
- Graph:** Sweep duration (ms) 10, 3D Graph.

17. Shorten the time interval between both stimuli by decreasing the **Delay (ms)** value of the second stimulus relative to the first (green box in the picture above) in the range from 7 to 3 ms. Why is the second action potential not generated below a certain value of time delay? Changing **Delay (ms)** parameter of second stimulus, find with an accuracy of 0.1 ms, the shortest stimulation interval for which the second action potential is still generated. This value represents the relative refractory period of the action potential. Complete point 3 of the form.
18. To test the absolute refractory period, change the intensity of the second stimulus to the maximum value (for **Stimulus 2** set the **Amplitude (µA)** to 400 - the red frame in the figure above; set the **Delay (ms)** to 7 - the green frame in the figure above). Shorten the time interval between both stimuli decreasing the **Delay (ms)** value of the **Stimulus 2** in the range of 7 to 2 ms. Find the shortest interval for which the second action potential is still being generated with an accuracy of 0.1 ms. This value represents the absolute refractory period of action potential. Complete point 3 of the form.

Fourth part

Aim: In this section, the relationship between the input resistance of a neuron and its ability to generate action potential will be examined.

The input resistance is approximately the reciprocal of the total conductivity of the entire membrane of the neuron. In our simulations, the input resistance will be represented by the reciprocal of the leakage conductivity (**gLeak**). This is a simplification that we take to avoid overcomplicating the simulation. The more ion channels remain open under given conditions, the greater the total conductivity of the membrane and the lower neuronal input resistance. By performing the simulation, we will answer the question: which neuron in a given structure of the brain more easily fire an axon potential, the one with high or low input resistance? This is the key question that a neurophysiologist asks when studying excitable cells.

Course of the simulations:

19. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut).
20. To run the simulation: extend the simulation window to 25 ms by setting the value 25 in the **Sweep duration (ms)** window; extend the duration of the depolarizing **Stimulus 1** to 24 ms by setting 24 in the **Width (ms)** window for Stimulus 1. We will examine the excitability of three neurons: with low, medium and high input resistance. Count how many action potentials will be elicited by the same stimulus applied to a neuron characterized by high input resistance (parameter **gLeak (mS/cm²)** with a value of 0.5), a cell with an average input resistance (parameter **gLeak (mS/cm²)** with a value of 4) and a cell with low input resistance (parameter **gLeak (mS/cm²)** with a value of 15). Knowing that the total conductivity (**gLeak** parameter) is the reciprocal of the input resistance, complete the table in point 4 of the form. How does the ability to generate action potentials change as the membrane input resistance increases (remember Ohm's law).

Fifth part

Aim: The aim of this section is to investigate how the change in potassium conductivity affects the course of the action potential

Course of the simulations:

21. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut).
22. The aim of this task is to investigate the impact of overexpression of voltage-dependent potassium channels on the course of action potential. In medicine, compounds that increase the activity of voltage-gated potassium channels are used, for example, in the pharmacotherapy of epilepsy. In the analyzed model, the total conductivity of the membrane for potassium ions is defined by the parameter **gK max (mS/cm²)**. The value of this parameter is proportional to the number of voltage-dependent potassium channels in the membrane of a given neuron. In order to increase total conductivity of the membrane for potassium, the parameter **gK max (mS/cm²)** should be set as: 50, 70, 100 mS/cm². Measure the duration and the latency of the obtained action potentials. Complete the table in point 5 of the form.
23. **Tetraethylammonium (TEA)** is a toxin that blocks the activity of voltage-gated potassium channels. Investigate how the application of different concentrations of TEA will affect the generation and kinetics of the action potential. In order to perform the simulation, the parameter **gK max (mS/cm²)** should be set as: 40, 25, 10 mS/cm² (the higher the TEA concentration, the less potassium channels are active, thus the lower total conductivity of the neuron membrane for potassium ions **gK max**). Measure the duration and latency of the obtained action potentials. Complete the second part of the table in point 5 of the form. Analyze the obtained data, briefly describe how changes in potassium conductivity affect the duration of action potential and neuronal excitability.

Sixth part

Aim: In this section, we will examine the effect of increased extracellular concentration of potassium ions on the course of action potential.

Course of the simulations:

24. Hyperkalemia is a pathological condition of excess potassium K⁺ in the blood serum, above the concentration of 5.5mM. The consequence may be muscle paralysis, arrhythmia and even cardiac arrest. Analyzing the Nernst formula, calculate equilibrium potential for potassium assuming three extracellular concentration of K⁺: the physiological $[C_{K^+}]_{out}=5.1\text{mM}$; moderate hyperkalemia $[C_{K^+}]_{out}=10\text{mM}$ and strong hyperkalemia $[C_{K^+}]_{out}=20\text{mM}$. Assume: intracellular concentration of potassium ions $[C_{K^+}]_{in}=115\text{mM}$ and temperature equal to 290 K. You may use, equation derived below. Fill the table in point 6 of the form.

$$V_{K^+_{eq}} = \frac{RT}{zF} \ln \frac{[C_{K^+}]_{out}}{[C_{K^+}]_{in}} = 2.3 \frac{RT}{zF} \log \frac{[C_{K^+}]_{out}}{[C_{K^+}]_{in}} = 58 \log \frac{[C_{K^+}]_{out}}{[C_{K^+}]_{in}} \quad [mV]$$

25. Simulate action potential for the calculated values of the equilibrium potential for potassium ions. Restore the default program settings by selecting the **File** tab and **Restore All to Default** from the drop-down menu (or use **Ctrl + D** shortcut); extend the simulation window to 12ms by setting the value 12 in the **Sweep duration**; then set the calculated values of the equilibrium potential in the **K+ equilib potential (mV)**.
 26. Why, in moderate hyperkalemia, spontaneous action potentials appeared without the application of a second stimulus? Hint: Notice how the resting membrane potential changes.
- FOR INTERESTED: Why is the action potential not generated under severe hyperkalemia? Hint: consider the mechanism of deinactivation of voltage-dependent sodium channels.

Required theoretical knowledge:

1. Nernst equilibrium; equilibrium potentials for sodium, potassium and chloride ions. Definition of electric driving force,
2. Basic concepts of electrodiffusion (migration - Ohm's law, diffusion - Fick's law, electric potential, electrochemical potential).
3. The origin of resting potential. Goldman-Hodgkin-Katz equation.
4. Voltage-gated ion channels: molecular structure, classification, biophysical properties. Voltage sensor, activation gate, inactivation gate.
5. Threshold potential and its dependence on membrane conductance for sodium, potassium and chloride ions.
6. Explanation of the time course of action potential on the basis of kinetic properties of voltage-gated ion channels.
7. Mechanism of action potential propagation in myelinated and non-myelinated axons; the role of inactivation of voltage-gated sodium channels.
8. Ion channel toxins (TTX and TEA) and their influence on action potential. Application in medicine.

Recommended literature:

1. R. Cotterill, Biophysics. An Introduction, Wiley 2004.
2. R. Glaser, Biophysics, Springer 2001.
3. <http://butler.cc.tut.fi/~malmivuo/bem/bembook/>
4. <http://www.st-andrews.ac.uk/~wjh/neurotut/mempot.swf>

Wroclaw Medical University Department of Biophysics and Neuroscience	Practical No 11 Computer simulation of action potential generation	
..... Students' names		Faculty: Group: Date:
Grade:	Tutor's signature	

1a. Plot the coordinate axes and waveform of the action potential generated for default parameters of simulation. Mark and sign characteristic parts axon potential time-course



Amplitude of axon potential [mV]			Duration of axon potential [ms]			Latency of axon potential [ms]		
V_m	V_{max}	$V_{max} - V_m$	t_d	t_r	$t_r - t_d$	$t_{stimulus}$	t_{Vmax}	$t_{Vmax} - t_{stimulus}$
-65 mV						0.5 ms		

1b. Why the tetrodotoxin blocks generation of axon potentials.

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2a. The impact of intensity of depolarizing stimulus on action potential generation.

Intensity of depolarizing stimulus [μA]	Amplitude of axon potential [mV]			Axon potential latency [ms]		
	V_m	$V_{\max}$	$V_{\max} - V_m$	t_{stimulus}	$t_{V_{\max}}$	$t_{V_{\max}} - t_{\text{stimulus}}$
50	-65 mV			0.5 ms		
100	-65 mV			0.5 ms		
150	-65 mV			0.5 ms		
200	-65 mV			0.5 ms		
Rheobase				μA		

2b. Does the intensity of the stimulus that excites the neuron affect the amplitude or latency of action potential?

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2c. The impact of duration of depolarizing stimulus on action potential generation.

Duration of depolarizing stimulus [ms]	Intensity of depolarizing stimulus [μA]	Does the neuron fires an axon potential YES/NO
0.1	10	
0.4	10	
0.8	10	
1.2	10	
The shortest duration of depolarizing stimulus eliciting an axon potential	10	ms

2d. Why does reaching the excitation threshold depend not only on the intensity of the depolarizing stimulus, but also on its duration?

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3. Analysis of relative and absolute refraction of the action potential

The shortest interval after which a second stimulus of the same amplitude generates an action potential [ms]	
The shortest interval after which the second stimulus with the maximum amplitude generates an action potential [ms]	

4a. Input resistance and the excitability of a neuron.

	g_{Leak} [mS/cm ²]	$1/g_{Leak}$ – input resistance [k Ω /cm ²]	Number of elicited axon potentials
Neuron with big input resistance	0.5		
Neuron with medium input resistance	4		
Neuron with small input resistance	12.5		

4b. How does the excitability of the cell depend on input resistance.

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5a. The dependency of action potential on membrane conductivity for potassium ions.

	gK_{max} (mS/cm ²)	Duration of axon potential [ms]			Latency of axon potential [ms]		
		t_d	t_r	$t_r - t_d$	$t_{stimulus}$	t_{Vmax}	$t_{Vmax} - t_{stimulus}$
Overexpression of potassium channels	50				0.5 ms		
	70				0.5 ms		
	100				0.5 ms		
Low concentration of TEA	40				0.5 ms		
Medium concentration of TEA	25				0.5 ms		
High concentration of TEA	10				0.5 ms		

5b. Why at high potassium conductivity, the axon potential is not generated

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5c. Describe the effect of TEA on axon potential time course.

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6a. Hyperkalemia and axon potential

	$[K^+]_{out}$ [mM]	$[K^+]_{in}$ [mM]	$V_{K^+ eq}$ [mV]
Physiological concentration	5.1	115	
Hyperkalemia	10	115	
Severe hyperkalemia	20	115	

6b. Why, in moderate hyperkalemia, spontaneous action potentials appeared without the application of a second stimulus? Hint: Notice how the resting membrane potential changes.

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6c. FOR INTERESTED: Why is the action potential not generated under severe hyperkalemia? Hint: consider the mechanism of deinactivation of voltage-dependent sodium channels.

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