PROPAGATION OF ACTION POTENTIAL ALONG UNMYELINATED AND MYELINATED AXONS

Aim of the practical

The aim of this practical is to examine the mechanism of action potential propagation along unmyelinated and myelinated axons. Relationship between velocity of action potential propagation and axon's diameter, spatial distribution of voltage-gated sodium and potassium channels and the number of turns of myelin sheath, will be examined.

Equipment

Computer software for simulation of electrophysiological measurements - „Neurons in Action".

The software contains following sections:

a) **Panel Manager** – main section, which manages display of additional sections, plots and windows
b) **Run Control** – section, which is responsible for starting simulations and setting parameters of the simulations,
c) **Stimulus Control** – section, which enables changes of stimulus parameters

Part IA Propagation of action potential along unmyelinated axon

Aim: studies on relationship between velocity of action potential propagation along an unmyelinated axon and axon's diameter.

Course of the Part IA:

1. Start the program **Neurons in action**. Choose the section **The unmyelinated axon** from the **Tutorials** menu. Run the simulation program by clicking the button **Start the Simulation**. Additional section - **Graph** (one window) – plot of membrane potential measured at one point in the axon vs. time, will appear.
2. In order to accomplish the goal of this part of the practical, do it according to the following points:

a) Set the temperature of simulation on the value of 20^o C by setting the proper value in the window next to the button **Temp [deg C]** in the section **Run Control.**
b) By clicking proper buttons in the section **Panel Manager** open additional windows: **Voltage vs Time – Quad Traces** and **Voltage vs Space.**
c) Run the simulation by clicking the button **Reset&Run** in the section **Run Control**. In order to better observe the simulations, set the monitor display rate on the minimal value by setting the horizontal indicator of the display rate in the section **Run Control** at the left extreme position (slower).
d) The window (**Graph[0]**) shows the plot of membrane potential measured at one point in the axon vs. time, the window (**Graph[1]**) shows the plot of membrane potential measured at four additional points in the axon vs. time, the third graph (**Graph[2]**) shows the plot of membrane potential vs. distance from the beginning of the axon at a given time. In order to better see the simulation, it is possible to change the scale by pressing the right button of the computer mouse and choosing the option **zoom in 10%** from the **View…** panel.
e) Investigate the influence of the axon's diameter on the velocity of action potential propagation. For this purpose, click the button **Axon parameters** in the section **Panel Manager.** Change the axon's diameter by clicking the button on the right side of the window **Diameter [µm]** in the section **Axon Parameters**, or by writing the new value down into the window. Introduce the values of 5, 10, 50, 100, 200 and 300 µm, one after another. Do the simulation of action potential propagation for all the diameters by clicking the button **Reset&Run** in the section **Run Control**. Calculate the velocity of action potential propagation for each diameter. For this purpose, enlarge the window **Graph[1].** By clicking the left button of the mouse place the cursor at the point of the maximal value of action potential at the point of measurement marked by the blue arrow (blue curve). Read the value of time of appearance of the maximum at this point – this is the x parameter. Write this value down to the Table no 1 into the box T_{Blue}. Then, by clicking the left button of the mouse place the cursor at the point of the maximal value of action potential at the point of measurement marked by the green arrow (green curve). Read the value of time of appearance of the maximum at this

point – this is the x parameter. Write this value down to the Table no 1 into the box T_{Green}. Calculate the difference T_{Green}–T_{Blue} and write this value down to the Table no 1 into the next box. Assuming the distance between the points marked by the arrows: blue and green, as equal to 3 mm, calculate the velocity of action potential propagation along the unmyelinated axon (v_{unmiel}), for each above-mentioned diameter. Write the results down into the Table 1 (point IA1 of the final report sheet). Plot the relationship of this velocity vs. axon's diameter (point IA2 of the final report sheet). Define mathematical relationship between velocity of action potential propagation along the unmyelinated axon and diameter of the axon.

3. Close all the windows in the section **The unmyelinated axon**. Go back to the menu **Tutorials.**

Part IB. Influence of spatial distribution of ion channels in an unmyelinated axon on action potential propagation

Aim: influence of changes of sodium and potassium conductance in a model unmyelinated axon containing three parts of the same length. During simulation, student can change both sodium and potassium conductance in the middle part of the axon. Following colour code was applied in the simulation: green - left part, red – middle part, blue – right part.

Course of the Part IB:

4. Choose the section **Non-uniform Channel Density** from the **Tutorials** menu. Run the simulation program by clicking the button **Start the Simulation**. Additional sections will appear:
   - **Graph** (two windows) – plot of membrane potential measured at one point in each part of the axon vs. time and a plot of sodium current intensity vs. time.
   - **Middle Axon Parameters –** section responsible for setting parameters of the middle part of axon.
5. In order to accomplish the goal of this part of the practical, do it according to the following points:

a) Close the window **Na currents vs time.**

b) By clicking proper buttons in the section **Panel Manager** open an additional window **Voltage vs Space**

c) Run the simulation by clicking the button **Reset&Run** in the section **Run Control.**

d) The window (**Graph[0]**) shows the plot of membrane potential measured at one point in each part of the axon vs. time, the window (**Graph[1]**) shows the plot of membrane potential vs. distance from the beginning of the axon at a given time. In order to better see the simulation, it is possible to change the scale by pressing the right button of the computer mouse and choosing the option **zoom in 10%** from the **View…** panel.

e) Investigate the influence of reduction of sodium conductance in the middle part of axon on the propagation of action potential. Reduce the sodium conductance in the range from standard value equal to 0.12 S/cm^2 to 0.01 S/cm^2 by clicking the button on the right side of the window **Na chan density [S/cm^2]** in the section **Middle Axon Parameters**, or by writing the new value down into the window. Do 4 simulations. Writing down values of the sodium conductance copy 4 plots of action potentials into proper frames – point IB1 of the final report sheet. Estimate a critical value of the sodium conductance, at which action potential propagation in the middle part of the axon is abolished – write the result down in the point IB1 of the final report sheet. Explain, why reduction of the sodium conductance in the middle part of axon below the critical value causes a decay of action potential in this part of axon.

f) Restore default value of sodium conductance by clicking the button on the left side of the window **Na chan density [S/cm^2]** in the section **Middle Axon Parameters**. Then, investigate the influence of increase of potassium conductance in the middle part of axon on the propagation of action potential. Raise the potassium conductance in the range from standard value equal to 0.036 S/cm^2 to 0.3 S/cm^2 by clicking the button on the right side of the window **K chan density [S/cm^2]** in the section **Middle Axon Parameters**, or by writing the new value down into the window. Do 4 simulations. Writing down values of the potassium conductance copy 4 plots of action potentials into proper frames – point IB2 of the final report sheet. Estimate a critical value of the potassium conductance, at which action potential propagation in the middle part of the axon is abolished – write the result down in the point IB2 of the final report sheet. Explain, why increase of the potassium conductance in the middle part of axon beyond the critical value causes a decay of action potential in this part of axon.

g) Restore default values of sodium and potassium conductance using buttons on the left of the window **Na chan density [S/cm^2]** and **K chan density [S/cm^2]**, respectively, in the section **Middle Axon Parameters**. Then, perform series of simulations decreasing the diameter of the middle part of axon from the standard value of 500 μm. Decrease the diameter from the standard value to 50 μm, by clicking the button on the right side of the window **Diameter [μm]** in the section **Middle Axon Parameters**, or by writing the new value down into the window. Do 4 simulations. The diameter 500 μm is a default value for all three parts of the axon. Only the value for the middle part of axon is changed. Students should observe velocity of action potential propagation and estimate the value of diameter, at which the action potential transfer from the middle to the right part of axon is abolished – write down the result and answer questions in the point IB3 of the final report sheet.

6. Close all the windows in the section **Non-uniform Channel Density**. Go back to the menu **Tutorials.**

Part II. Propagation of action potential along myelinated axon

Aim: studies on relationship between velocity of action potential propagation along a myelinated axon and thickness of the myelin sheath.

Course of the Part II:

7. Choose the section **The myelinated axon** from the **Tutorials** menu. Run the simulation program by clicking the button **Start the Simulation**. Additional section - **Graph** (one window) – plot of membrane potential measured at one Node of Ranvier in the axon vs. time, will appear.
8. In order to accomplish the goal of this part of the practical, do it according to the following points:

a) By clicking proper buttons in the section **Panel Manager** open additional windows: **Voltage vs Time – Dual Traces** and **Voltage vs Space.**

b) Run the simulation by clicking the button **Reset&Run** in the section **Run Control**.

c) The window (**Graph[0]**) shows the plot of membrane potential measured at one Node of Ranvier in the axon vs. time, the window (**Graph[1]**) shows the plot of membrane potential measured at two additional Nodes in the axon vs. time, the third window (**Graph[2]**) shows the plot of membrane potential vs. distance from the beginning of the axon at a given time. In order to better see the simulation, it is possible to change the scale by pressing the right button of the computer mouse and choosing the option **zoom in 10%** from the **View…** panel.

d) Investigate the influence of the thickness of the myelin sheath on the velocity of action potential propagation. For this purpose, click the button **# Wraps of Myelin** in the section **Panel Manager.** Change the number of wraps by clicking the button on the right side of the window, or by writing the new value down into the window. Reduce the number of wraps from the standard value of 150 to 125, 100, 75, 50, 25 and 10, one after another. Do the simulation of action potential propagation for all the numbers, including the standard one, by clicking the button **Reset&Run** in the section **Run Control**. Calculate the velocity of action potential propagation for each number, except for the last one. For this purpose, click on the button **Total#[ms]** in the section **Run Control** and increase the time of simulation from 1.5 ms to 2ms by clicking the button on the right side of the window. Then, enlarge the window **Graph[1].** By clicking the left button of the mouse place the cursor at the point of the maximal value of action potential at the Node of Ranvier marked by the red arrow (red curve). Read the value of time of appearance of the maximum at this point – this is the x parameter. Write this value down to the Table no 2 into the box T_{Red}. Then, by clicking the left button of the mouse place the cursor at the point of the maximal value of action potential at the Node of Ranvier marked by the black arrow (black curve). Read the value of time of appearance of the maximum at this point – this is the x parameter. Write this value down to the Table no 2 into the box T_{Black}. Calculate the difference $T_{Black}–T_{Red}$ and write this value down to the Table no 2 into the next box. Assuming the distance between the Nodes of Ranvier marked by the arrows: red and black, as equal to 8 mm, calculate the velocity of action potential propagation along the myelinated axon (v_{myel}), for each above-mentioned number of wraps. Write the results down into the Table 2 (point II1 of the final report sheet). Define the reason of influence of decrease of the number of wraps on the velocity of action potential propagation along myelinated axon. Plot the relationship of this velocity vs. number of wraps (point II2 of the final report sheet). Explain the reason of lack of action potential at the number of wraps equal to 10 (point II3 of the final report sheet).

e) After having finished the simulations close all the windows in the section **The myelinated axon.** Then, close the program **Neurons in action**.

Required theoretical knowledge

1. Passive electrical properties of cell membranes: electrical resistance and capacitance of membrane, membrane time and space constant, equivalent electrical circuit of a membrane.
2. Mechanism of action potential generation in a neuron (explain the phenomenon of threshold)
3. Propagation of action potential along unmyelinated and myelinated axons.

Literature

1. Pronciples of Neural Science, Kandel E., McGraw-Hill Education, 5th edition 2012
2. Cotterill R. Biophysics. An introduction. J. Wiley & Sons, 2004.
3. Glaser R. Biophysics an introduction. 2-nd ed. – Berlin: Springer, 2012.

Medical University in Wrocław Department of Biophysics and Neuroscience	Practical 16 **Propagation of action potential along unmyelinated and myelinated axons**

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Grade:	Tutor's signature

Part IA

IA1. Table 1.

Diameter of unmyelinated axon [μm]	**T_{Blue} [ms]**	**T_{Green} [ms]**	**$T_{Green} - T_{Blue}$ [ms]**	**V_{unmyel} [m/s]**
5				
10				
50				
100				
200				
300				

IA2 Add the plot of relationship: **v_{unmyel} vs. diameter of unmyelinated axon**. Define mathematical relationship between velocity of action potential propagation along the unmyelinated axon and diameter of the axon:

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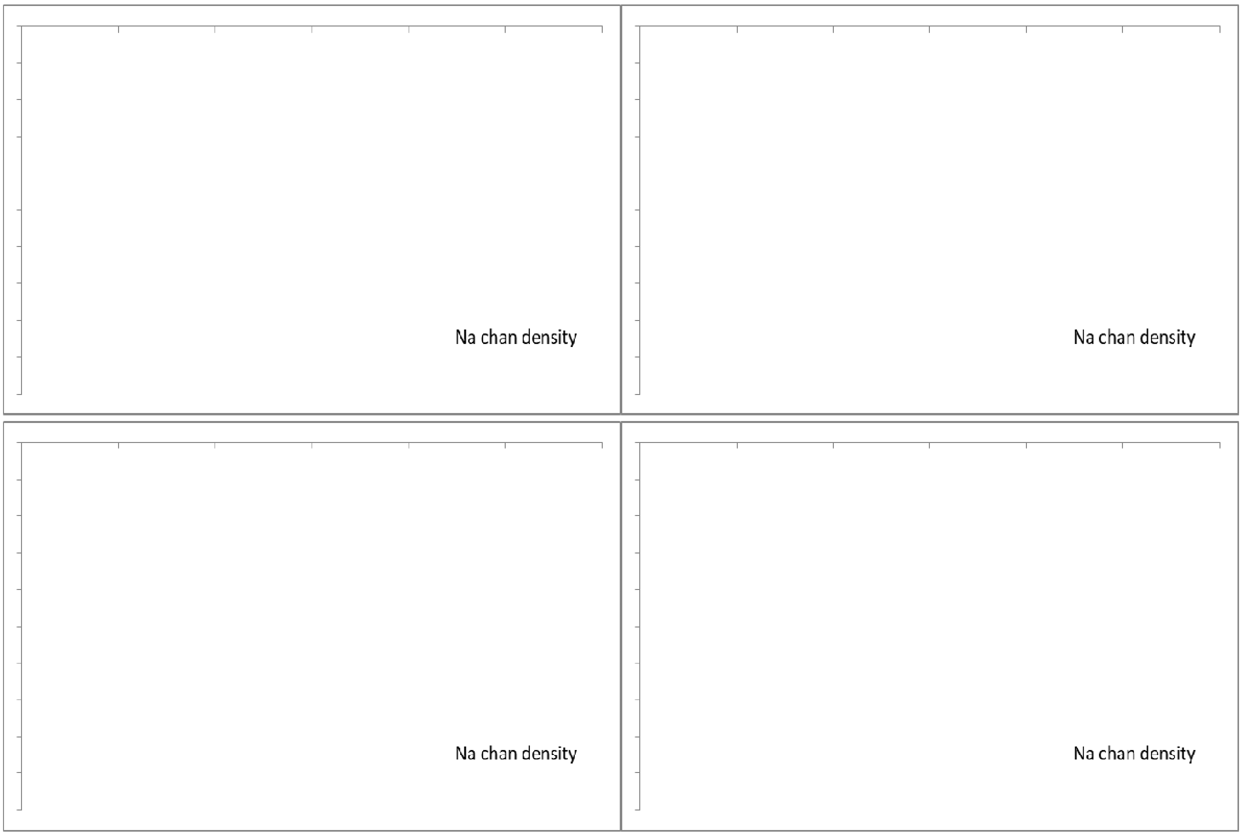
Part IB

IB1. Copy the plots of action potential writing down the values of sodium conductance (**Na chan density [S/cm^2]**).



Critical value of **Na chan density**

Explain, why reduction of the sodium conductance in the middle part of axon below the critical value causes a decay of action potential in this part of axon:

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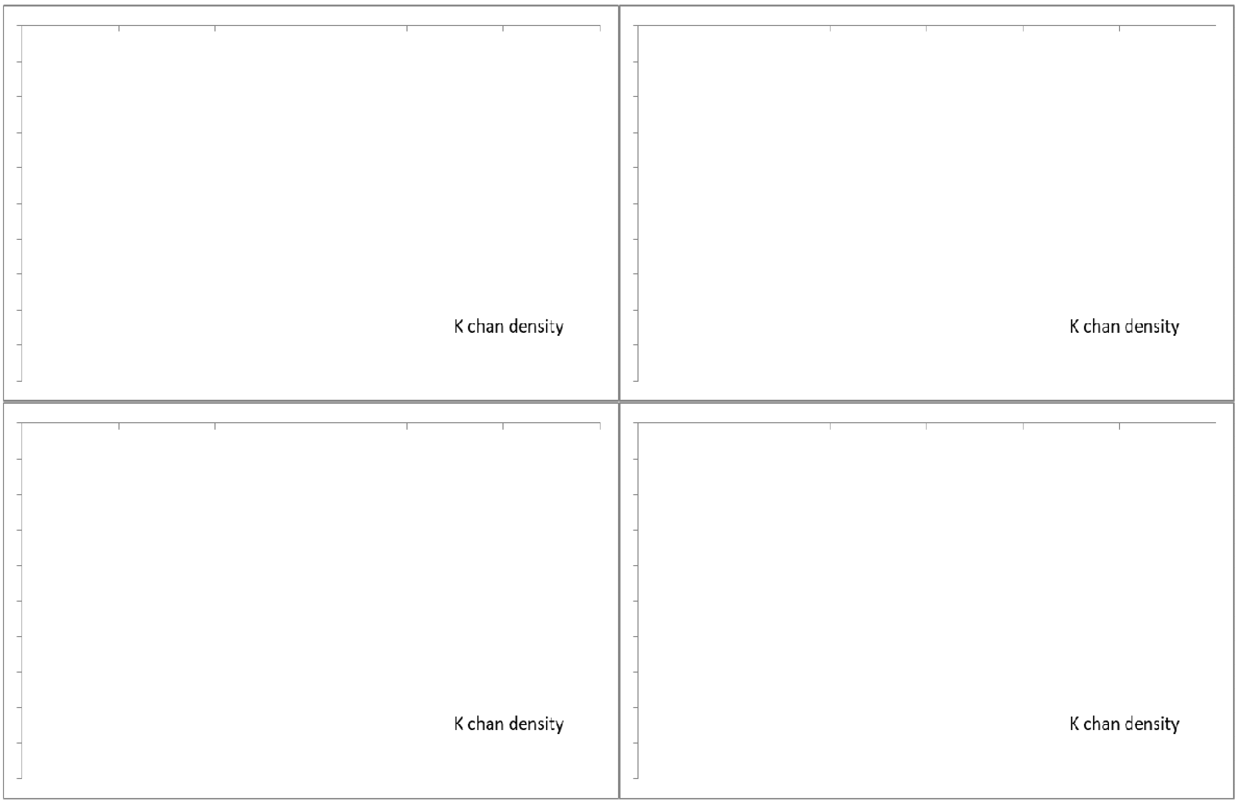
IB2. Copy the plots of action potential writing down the values of potassium conductance (**K chan density [S/cm^2]**).



Critical value of **K chan density**

Explain, why increase of the potassium conductance in the middle part of axon beyond the critical value causes a decay of action potential in this part of axon:

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IB3. Under which circumstances the action potential transfer from the middle to the right part of the axon is abolished ?

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IB3. Explain, why an action potential, generated as usual in the middle part of the axon, is NOT transferred to the right part:

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Part II

II1. Table 2

Number of wraps of myelin sheath	T_{Red} [ms]	T_{Black} [ms]	$T_{Black} - T_{Red}$ [ms]	v_{myel} [m/s]
150				
125				
100				
75				
50				
25				

II2. Add the plot of v_{myel} **vs. number of wraps of myelin sheath**. Define the reason of influence of decrease of the number of wraps on the velocity of action potential propagation along myelinated axon:
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II3. Explain the reason of lack of action potential at the number of wraps equal to 10.

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