

ANALOG MODEL OF SYNAPTIC TRANSMISSION

Synaptic transmission is one of the most important processes that occur in living organisms. Studying synaptic transmission under natural conditions is not possible in this laboratory. However, it is possible to employ an analog model to simulate synaptic transmission and study relationships between the changes of neuron's membrane potential and the magnitude of current stimulus and between the electrical activity of a neuron and the magnitude of depolarisation of neuron's body. During this practical the process of charging the membrane capacitor by electric current generated by activation of a synaptic connection is also studied

A more detailed physical description of this process is shown in Figure 1A. Each biological membrane is a capacitor with electric capacitance (C_m) and a resistor with electric resistance (R_m). Moreover, each membrane has its own electric membrane potential (V_m) equal to the electric potential at the inner side of membrane minus the potential at the outer side. When the electric connection is disrupted (Fig. 1A), no electric current is flowing through the membrane model – this corresponds to physiological situation when the synapse is not active. In such a case, the membrane potential V_m is equal to its resting value V_r .

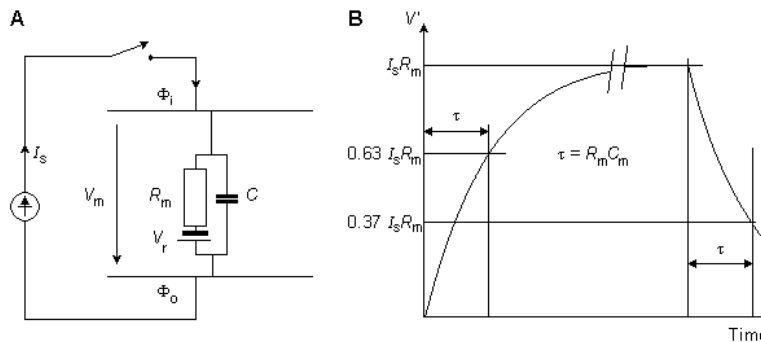


Figure 1.

When the electric connection is working (Fig. 1B), the current I_s is flowing through the membrane model – this corresponds to physiological situation when the synapse is working. Part of this current is flowing through the resistor and part through the capacitor charging its electrical capacitance. According to the Ohm's law, passing of electric current through the membrane resistor changes its membrane potential V_m , but this change does not occur immediately - it requires time to charge the electrical capacitance C_m . The time needed to change the V_m to $(1-1/e)$, it means to about 63% of its final value, is called the membrane time constant τ (greek consonant „tau”). When the current I_s is turned off the time constant defines time needed to change the V_m to $(1/e)$, it means to about 37% of its initial value (Fig. 1B). In both cases the time constants are equal to each other and equal to $R_m \cdot C_m$ (Fig. 1B). The units of measure of a time constant are usually milliseconds [ms] ($1 \text{ ms} = 0.001 \text{ s}$).

DESCRIPTION OF THE EXPERIMENTAL SETUP

In this practical we use an analog model of the synaptic transmission, analog-to digital converter and a computer with recording and analysing software.

Experimental setup contains the following parts:

- a. Operating Unit for Neurosimulator is a source of electric signals that activate synaptic connections.

Generation of such a signal corresponds to neurotransmitter release in a living organism.

- b. Neurosimulator – is a main part of the model. All model's parts are shown on the front panel:

- neuron's cell body and its dendrites
 - six synaptic connections formed with the neuron's cell body or its dendrites (three of them are additionally modulated by axon-axon synapses)
 - myelinated axon of the neuron
- c. Analog-to-Digital Converter (Basic Unit Cobra 3)
- d. Computer with installed software – „Measure” – Program

The Operating Unit, Neurosimulator and Analog-to-Digital Converter (all parts with numbered buttons, inputs and outputs) are shown in the attached photo.

Pressing any of the buttons 4-6 of the Operating Unit causes generation of electric signal that corresponds to neurotransmitter release in a living organism. The signal is generated as long as the buttons remain pressed. The intensity of the signal can be modulated by the regulators 1-3, which are located above the appropriate buttons. The generated signal is released from the Unit by the outputs 7-9.

To transmit the signal generated by the Unit to the Neurosimulator one has to connect outputs of the Unit to the inputs of the Neurosimulator (10-18) by cables.

Synaptic inputs 11, 13 and 15 of the Neurosimulator correspond to excitatory synapses (green colour), whereas inputs 17 and 18 correspond to inhibitory synapses (red colour).

Outputs of the Neurosimulator contain information about membrane potential of the neuron's cell body (19) and the axon's membrane potential measured in Node of Ranvier (20). When action potentials are generated in the axon a sound signal is additionally generated by the Neurosimulator.

The values of membrane potentials in the Neurosimulator are much higher than the values in any real neuron.

For example, the membrane resting potential in case of a typical neuron is equal to about -70 mV, whereas in case of the Neurosimulator it is equal to about -6.9 V. This is because the level of background noise is much higher in the Neurosimulator than in a real living neuron. To obtain the same signal-to-noise ratio one has to increase the membrane potential respectively.

The membrane potentials in the outputs of the Neurosimulator must be sent to the Analog-to-Digital Converter to be converted to digital signals and then sent to a computer to be measured and analysed by the software. For this purpose, one has to connect the Neurosimulator's outputs (19) and/or (20) to the converter's inputs (21) and/or (22) with cables.

COURSE OF MEASUREMENTS AND DATA DISPLAY

PART A. CALCULATION OF THE MEMBRANE TIME CONSTANT

Preliminary operations:

- a) turn the computer on
 - b) turn the Neurosimulator on (the power button is located in the upper part of the back panel of the device)
 - c) start the „Measure” computer program
1. Connect with cables: output (7) to the input (11) and output (19) to the input (21).
 2. Set the electric stimulus intensity to the maximal value using the regulator (1). The regulator's central line must be positioned in the right maximum.
 3. Click the option „new measurement” in the computer's program „Measure” (click red dot in the upper

- left corner of the program menu).
4. a) choose the option „normal measurement”
 - b) activate the first (left) analog channel in the window „channels”
 - c) choose „time” in the window „record X”
 - d) choose option „ $\pm 10V$ ” in the window „range”
 5. Click the option „continue” to go to the next dialogue window. Choose the option „start measurement” and, after a while, press the button (4) for about 3 seconds. After having released the button (4) click the option „stop measurement”.
 6. When the measurements are finished the plot of the membrane potential as a function of time (red curve) will appear on the screen. To calculate the time constant activate the function „survey”, indicated by the # symbol. Then, two points of measurements: „1” and „2” will be displayed on the screen.
 - a) using the computer’s mouse move the point „1” to the point of start of membrane depolarisation and the point „2” to the point of end of membrane depolarisation.
 - b) read the maximal value of the membrane potential’s change upon depolarisation - ΔY .
 - c) calculate the value of $0.63 \times \Delta Y$, which corresponds to the membrane potential’s change at the time equal to the time constant.
 - d) change the position of the point „2” from the previous position to the position, at which the ΔY is equal to the calculated $0.63 \times \Delta Y$ value.
 - e) read the ΔX value corresponding to the $0.63 \times \Delta Y$ value. This is the value of the time constant.
 - f) write down the value of the time constant to the Table 1 in the report’s sheet.
 7. Repeat the measurements from the points 3, 5 and 6 twice more for two other, significantly different, values of the electric stimulus intensity set by the regulator (1).
 8. Calculate the average value of the time constant and write down this value to the Table 1 in the report’s sheet.

PART B. STUDYING RELATIONSHIP BETWEEN THE AMPLITUDE OF MEMBRANE DEPOLARISATION AND INTENSITY OF ELECTRIC STIMULUS

1. Connect additionally the output (7) to the input (22) using a cable without disrupting other connections.
2. Using the regulator (1) set the stimulus intensity to its minimal value, which corresponds to the regulator’s central line position at the beginning of the scale above.
3. Click the option „new measurement” in the „Measure” Program. Then activate the second analog channel in the window „channels”.
4. Repeat the measurements described in points 4 and 5 of the section PART A to record the electric stimulus intensity and the membrane potential simultaneously.
5. When the measurements are finished the plot of the membrane potential as a function of time (red curve) and the plot of the electric stimulus intensity as a function of time (blue curve) will appear on the screen. To measure the amplitude of membrane depolarisation (red curve) activate the function „survey”, indicated by the # symbol. Then, two points of measurements: „1” and „2” will be displayed on the screen.
 - a) using the computer’s mouse move the point „1” to the point of start of membrane depolarisation and the point „2” to the point of end of membrane depolarisation.
 - b) read the amplitude of the membrane depolarisation - ΔY .
 - c) write down the value of the ΔY to the Table 2 in the report’s sheet.
6. To measure the intensity of electric stimulus click the option „U1”, then activate the function „measure”. Two points of measurements: „1” and „2” will be displayed on the screen.
 - a) using the computer’s mouse move the point „1” to the point of start of stimulus application and the point „2” to the point of end of stimulus application.

- b) read the value of the stimulus intensity - ΔY .
 - c) write down the value of the ΔY to the Table 2 in the report's sheet.
7. Repeat all these measurements for eight other increasing values of the electric stimulus intensity set by the regulator (1).
 8. Plot the relationship between the amplitude of membrane depolarisation and the value of the stimulus intensity on a plotting paper.

PART C. STUDYING RELATIONSHIP BETWEEN THE FREQUENCY OF GENERATION OF ACTION POTENTIALS IN THE AXON AND THE AMPLITUDE OF MEMBRANE DEPOLARISATION

1. Disconnect the cable from the output (7) and connect it to the output (20). Do not disrupt other connections.
2. Choose the option „new measurement” in the „Measure” program.
3. Choose the option „fast measurement”.
4. Save the measurement range of the upper analogous channel ($\pm 10V$), whereas the measurement range of the lower analogous channel must be set to $\pm 0.1V$.
5. Set the sampling frequency to 10 kHz.
6. Click the button „continue”. Then, a new window with a comment „wait for trigger” will appear.
7. Using the regulator (1) set the stimulus intensity to its minimal value, which corresponds to the regulator's central line position at the beginning of the scale above.
8. Press the button (4) for about 1 second. In the „fast measurement” mode pressing the button will automatically start recording and display of the membrane potentials by the software.
9. When the measurements are finished the plot of the membrane potential of the neuron's cell body as a function of time (red curve) and the plot of the axon's membrane potential measured in a Node of Ranvier as a function of time (blue curve) will appear on the screen. To measure the amplitude of the membrane cell body depolarisation (red curve) activate the function „survey”, indicated by the # symbol. Then, two points of measurements: „1” and „2” will be displayed on the screen.
 - a) using the computer's mouse move the point „1” to the point of start of membrane depolarisation and the point „2” to the point of end of membrane depolarisation.
 - b) read the amplitude of membrane depolarisation - ΔY .
 - c) write down the value of the ΔY to the Table 3 in the report's sheet.
10. Measure the frequency of generation of action potentials in the axon (f) - from the plot of the axon's membrane potential measured in a Node of Ranvier as a function of time (blue curve). For this purpose click the option „U1” and count the number of action potentials (N) generated in the time period ($T_L - T_F$) - where T_L is time of generation of the last action potential counted, T_F - time of generation of the first action potential counted. Since it is aimed to find the frequency of action potentials generation in the stationary state of a neuron, the value of T_F should be higher than the value of the membrane time constant found in PART A. Now, measure the value of $T_L - T_F$ using the function „survey”. After activation of this function, two points of measurements: „1” and „2” will be displayed on the screen.
 - a) using the computer's mouse move the point „1” to the T_F time and the point „2” to the T_L time on the X axis.
 - b) read the value of $T_L - T_F$ equal to ΔX .
 - c) calculate the value of the frequency applying the formula:

$$f = (N-1)/(T_L - T_F)$$

The unit of measure of the frequency is Hertz [Hz]. 1 Hz = 1/s

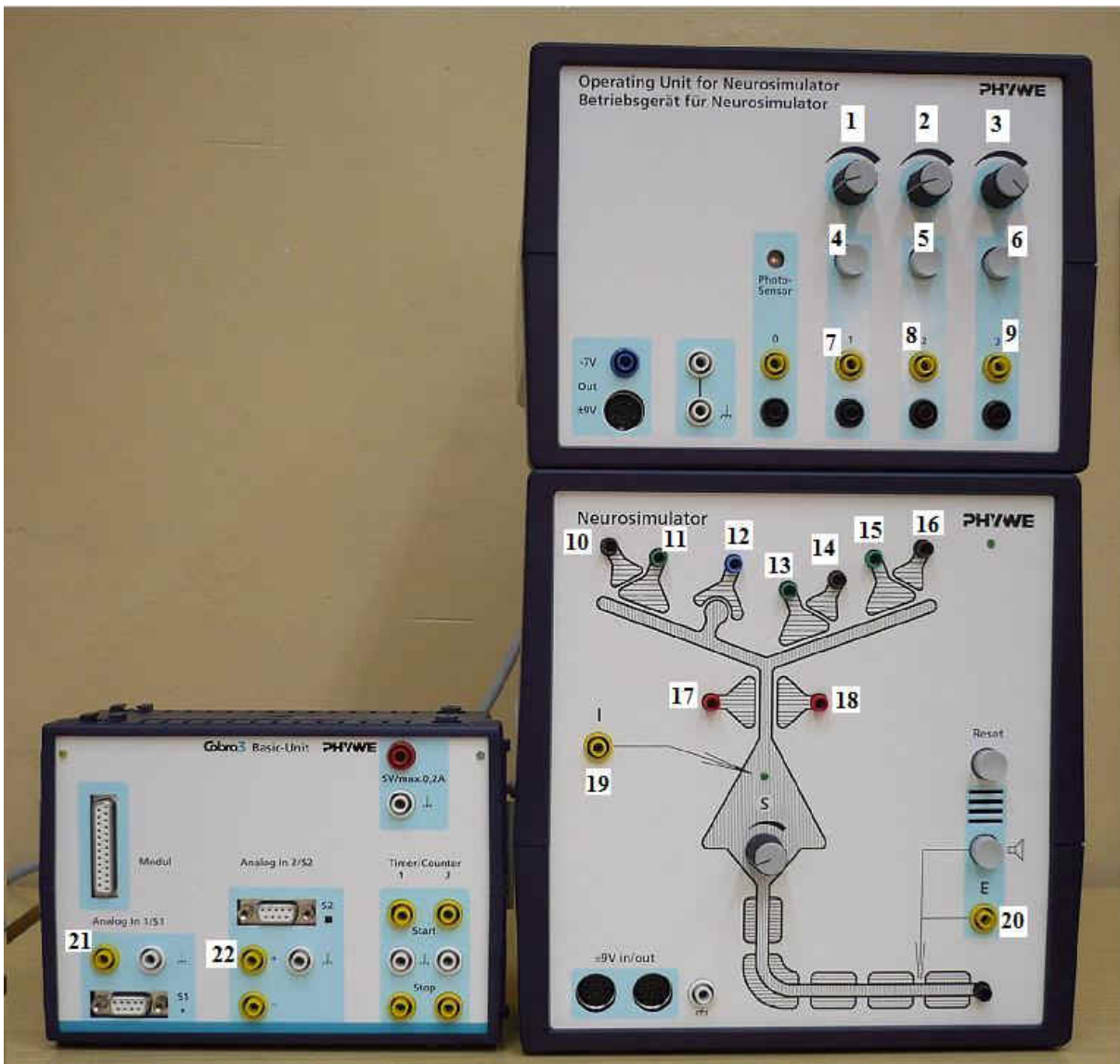
- d) write down the value of the f to the Table 3 in the report's sheet.
11. Repeat all these measurement for eight different rising values of the electric stimulus intensity set by the regulator (1).
 12. Plot the relationship between the frequency of generation of action potentials in the axon and the amplitude of membrane depolarisation.

Required theoretical background:

1. Passive electrical properties of cell membranes: electrical capacitance, electrical resistance, membrane time constant.
2. Action potential – basic properties and the mechanism of generation.
3. Structure of synapses between neurons.
4. Mechanism of conversion of electrical signal to chemical signal (neurotransmitter release) in the presynaptic ending.
5. Mechanism of conversion of chemical signal to electrical signal in the postsynaptic ending. Excitatory and inhibitory synapses.
6. Model of electric properties of a cell membrane and relationship between changes of a membrane potential and intensity of electric current flowing through a membrane.

Recommended literature:

1. Cotterill R. *Biophysics. An introduction*. J. Wiley & Sons, 2004.
2. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J. D., *Molecular Biology of the cell*, Garland Publishing, Inc., New York, 1994.
3. Glaser, R., *Biophysics*. Springer-Verlag, 2004.



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..... Names of students	Faculty: Group No: Date:
Grade:	Tutorial signature

Table 1. Measurement of the time constant of charging the membrane.

Measurement number	Membrane time constant [ms]
1	
2	
3	
Average value	

Table 2. Relationship between the amplitude of membrane depolarisation and intensity of current stimulus.

Intensity of electric stimulus [V]	Amplitude of membrane depolarisation [V]

Plot the dependence of the amplitude of membrane depolarisation on stimulus intensity.

Table 3. Relationship between the frequency of generation of action potentials in axon and the amplitude of depolarisation.

Amplitude of membrane depolarisation [V]	Frequency of action potential generation in the axon [Hz]

Plot the dependence of the frequency of generation of action potentials in the axon on the amplitude of membrane depolarisation.