SimNeuron

PROTOCOL FORMS for EXPERIMENTS in the CURRENT-/VOLTAGE-CLAMP LAB

1. Basic Current-Clamp Experiments: Action Potentials, Local Potentials and Passive Responses

For the following experiments we recommend to apply a short current pules, e.g. of 0.2ms, and to keep the pulse duration constant. We also recommend to plot the following recordings in one diagram (activate the "save" button).

1.1 Determine the current threshold for **action potential** generation.

pulse duration: ……. (ms) pulse amplitude …….. (nA)

1.2 Reduce the current amplitude to a value slightly below the threshold of AP generation to see **the "local" potentials.**

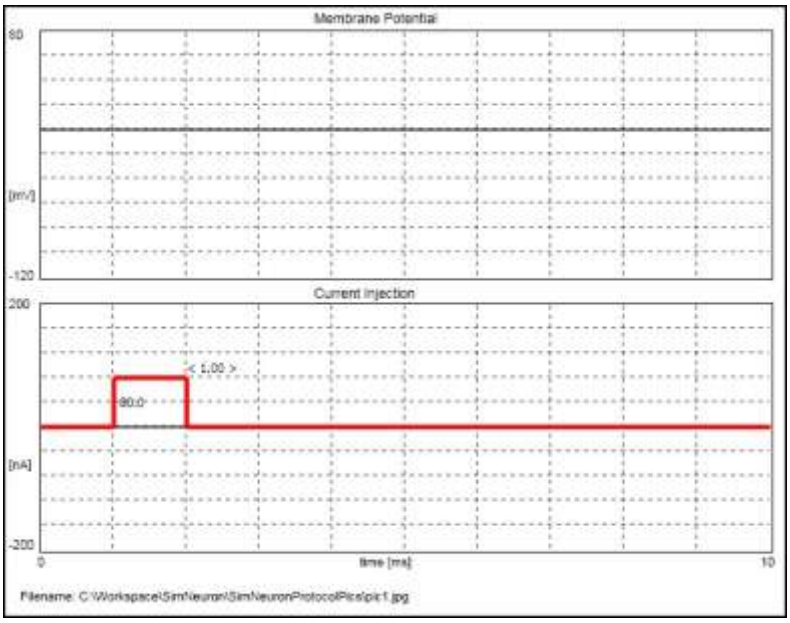
pulse amplitude …….. (nA)

1.3 Go back to the same stimulus amplitude as in 1.1. but apply TTX to prevent induction of active currents to see the pure **"passive" response**.

1.4 Apply the same stimulus in hyperpolarizing direction. Then switch off the TTX-application which should make no difference because **hyperpolarizing stimuli** do not activate potential dependent currents.

…here is space to insert your recordings (don't forget to document your results):

you know, you can copy your actual screen pressing the "PRINT" button of your keyboard and then can paste it directly into this program or into graphic programs like Photoshop which also allow to cut out the parts of interest, e.g. the diagrams.



2. The Strength-Duration Curve, Time Delays, Rheobase and Chronaxy

2.1 Determine Rheobase and Chronaxy and document the recordings.

Rheobase …… ……

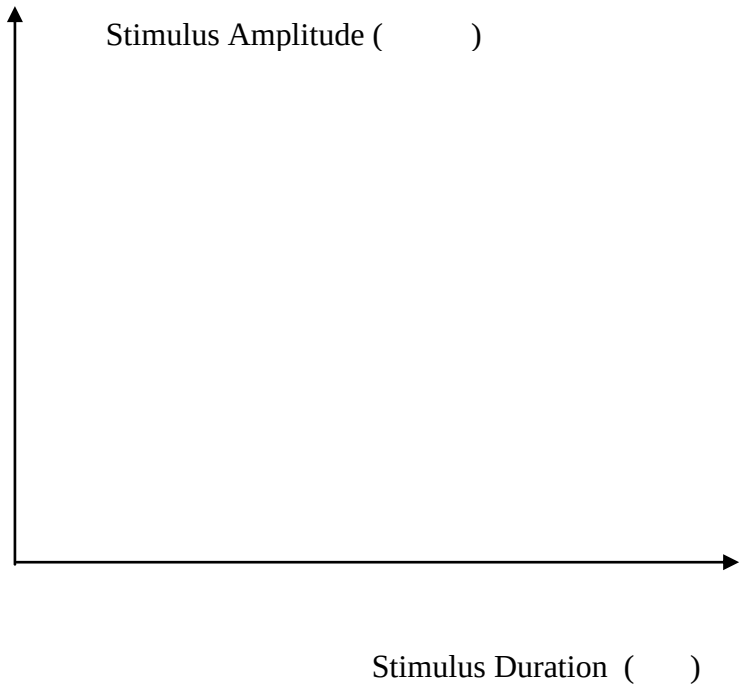
Chronaxy …… …….

2.2 Record the Strength-Duration Curve:

Table:

Stimulus Duration ( )	Stimulus Amplitude ( )

Diagram:



Please, care for an appropriate scaling of the axis to see the relevant range of the interdependencies of stimulus amplitude and duration.

Question:

Which are the membrane properties that are responsible for the time dependencies of action potential generation?

3. The Passive Membrane Proporties: Resistance and Capacitor (RC, τ)

To examine the pure passive responses and to be sure that not potential dependent currents are activated you can block all active currents of this neuron with **application of TTX and TEA**.

Then you can apply a strong and long-lasting current pulse which only induces passive potential changes (see also cLabs/membrane properties/RC-circuit and RC-lab).

Your Tasks:

3.1 Please apply a current pulse with an amplitude of about **50 nA** which lasts long enough (about **4 ms**) to allow complete charge of the membrane and which is positioned (between 2 and 6 ms) that you also can see the discharge.

We recommend to enhance the resolution of the membrane potential recording to a range from **–80 to –30 mV** which will make it easier to determine the **time constant** of the membrane.

3.2 Determine the membrane time constant τ: τ = ……

3.3 How is the time constant τ related to resistance R and capacitance C? τ = ……

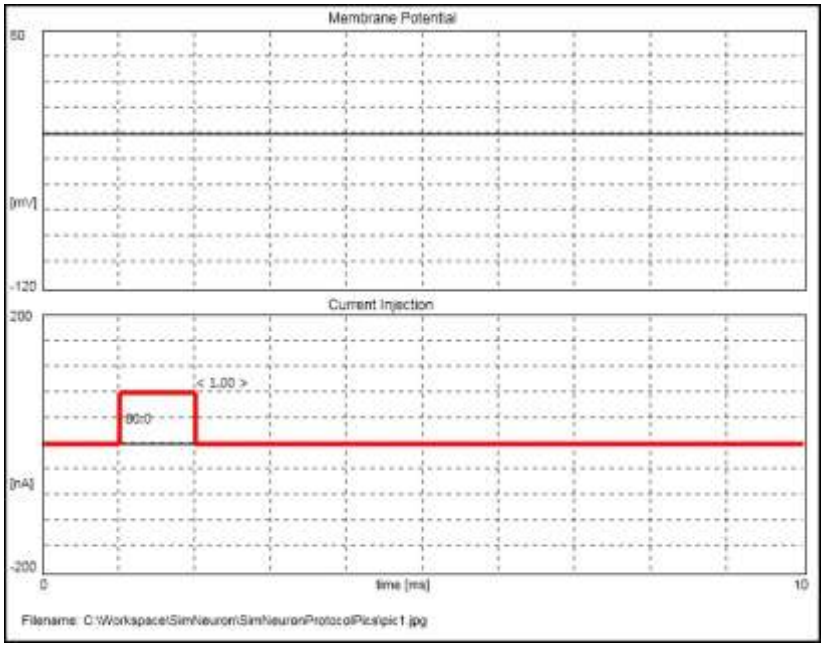
3.4 Calculate from your recording the resistance R and capacitance C: R = ……

C = ……

3.5 Please, additionally record, in the same diagram, the passive membrane responses when a very short current pulse of **0.2 ms** is applied.

3.6 Please indicate which part of recording 1 matches the repolarization of recording 2.

… space for your recordings:



Membrane Conductances and Currents

Document a recording in the current-clamp lab which shows the action potential together with the changes of Na- and K-conductances and –currents (activate the corresponding check boxes above the diagrams)

Try to explain the different phases of the response:

- Passive depolarisation, upstroke, downstroke and after-hyperpolarization
- Compare the time course of the membrane potential, conductances and currents. (remember Ohms law, "driving force")

Questions:

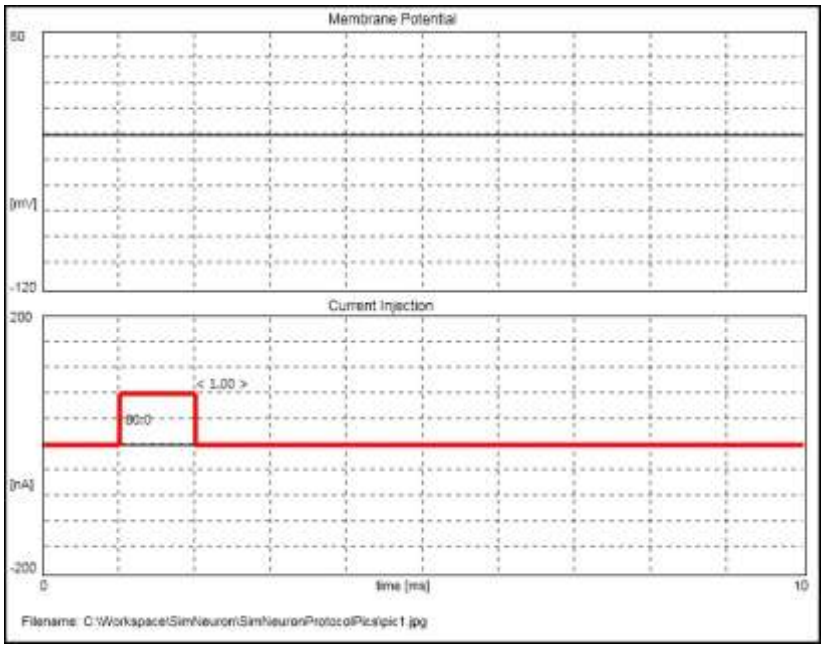
Eventually you might leave the following questions open until you have done the voltage-clamp experiments.

Try to explain the different phases of the response: initial depolarisation, fast upstroke, downstroke and after-hyperpolarization.

Write Ohms law in terms that descibe the interrelations between ionic currents, conductances and the relevant membrane potentials (remember the "driving force").

Use this formula to explain the different time course of conductances and currents, specifically why the Na-current transiently decreases during the action potential and why the K-current goes to almost zero during after-hyperpolarization.

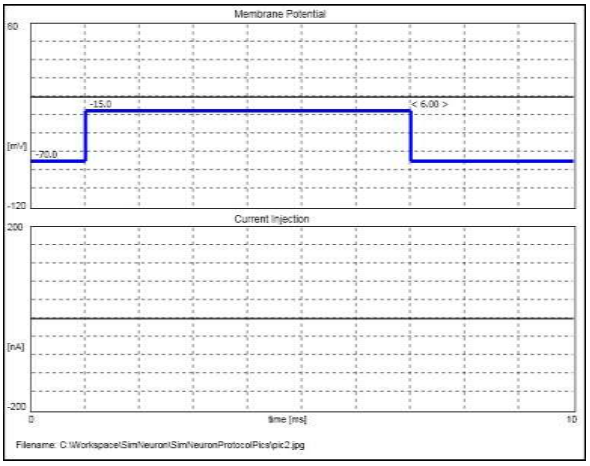
space for your recordings and comments:



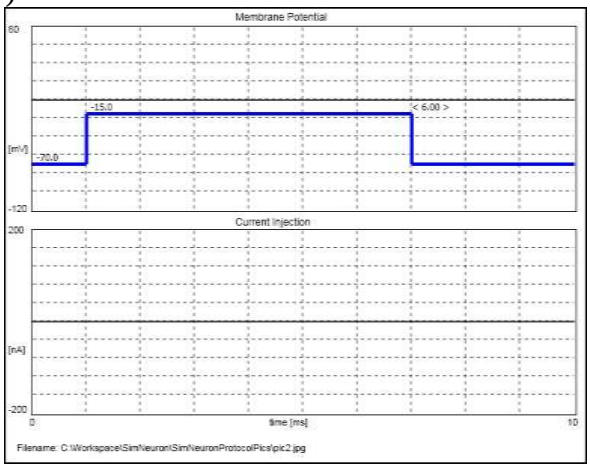
5. Basic Voltage-Clamp Experiments:

a) Please document a voltage-clamp recording without RC compensation. Your recording should elucidate all relevant characteristics: the passive current peaks at the beginning and the end of the stimulus as well as clear downward and upward deflections of active currents in between.
b) Please apply the same stimulus again but with activated RC-compensation.

a)



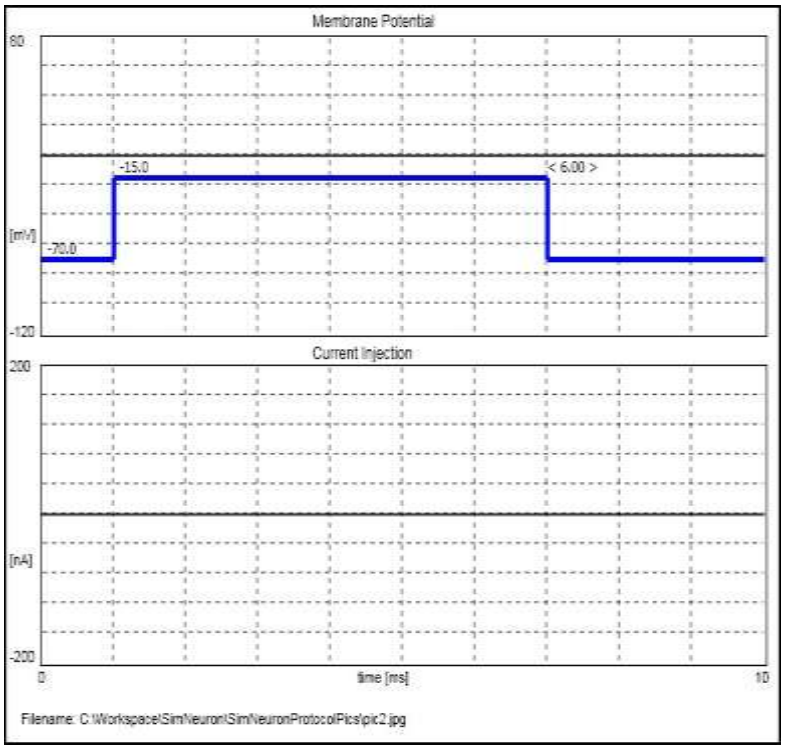
b)



Compare the effects of TTX and TEA application. Use the same settings as before and plot both recordings in one diagram (use the SAVE button). You also might enhance the resolution of the current plot (range –100 to +100).

Additional tasks:

a) Please try to reconstruct the total current curve. Herefore, please mark the total currents at 0.5, 1 and 2 ms after stimulus onset.
b) Can you describe the major differences between Na- and K-currents concerning the time-dependencies of activation and eventual inactivation.
c) Please try to estimate the different time-constants. In case of Na-activation you should enhance the time-scale.



K: τ_n = ……...

Na : τ_m = ……...

τ_h = ……

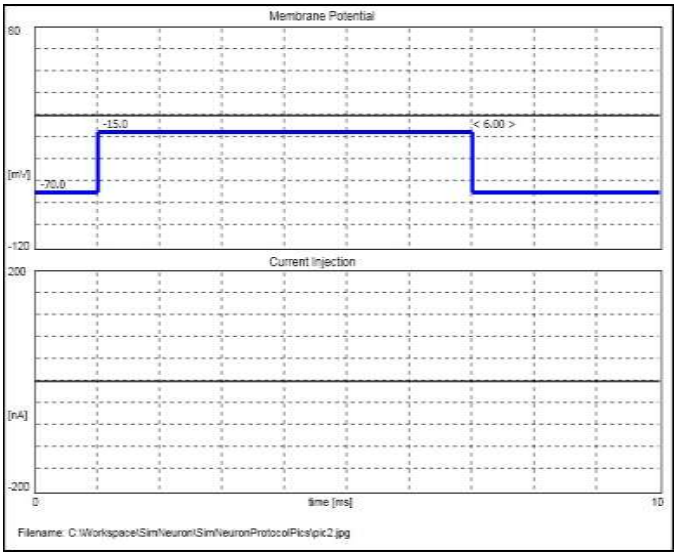
6. Voltage-Dependencies of Na^+-and K^+-currents:

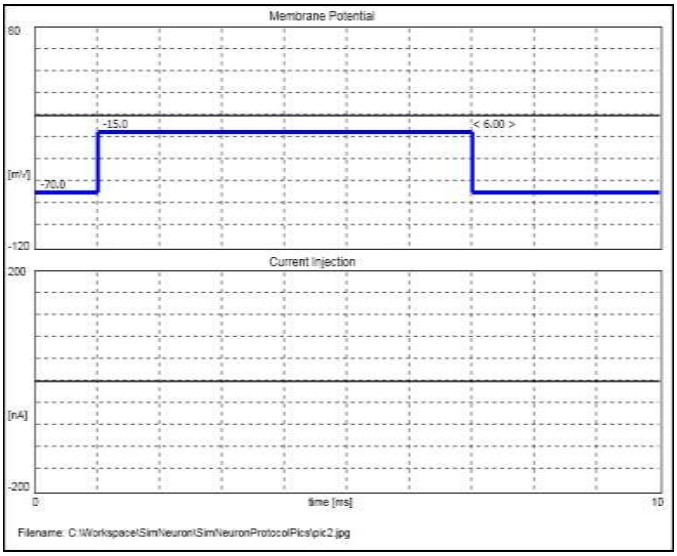
Apply a complete "family" of command voltages (different stimulus amplitudes) to see how the current curves change.

Then, please, do the same experiments again with

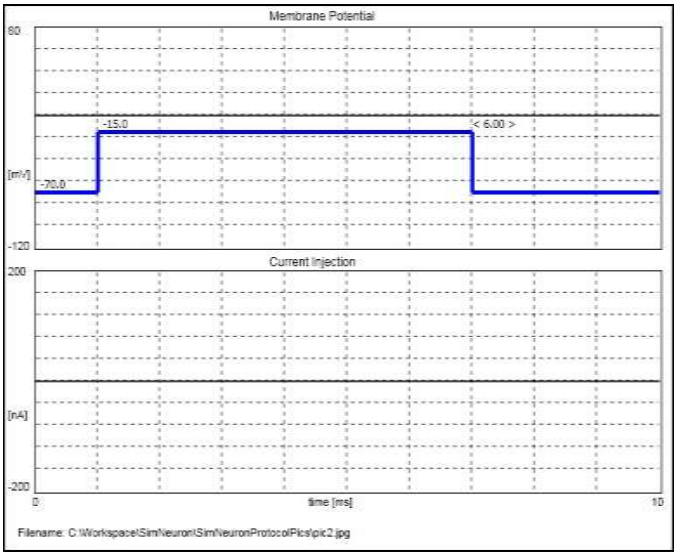
a) application of TTX and
b) application of TEA.

Can you explain why the amplitude of the K-current increases monotonically with increasing command voltages whereas the maximum value of Na-currents first increase and then decreases and then even turn into an opposite direction?





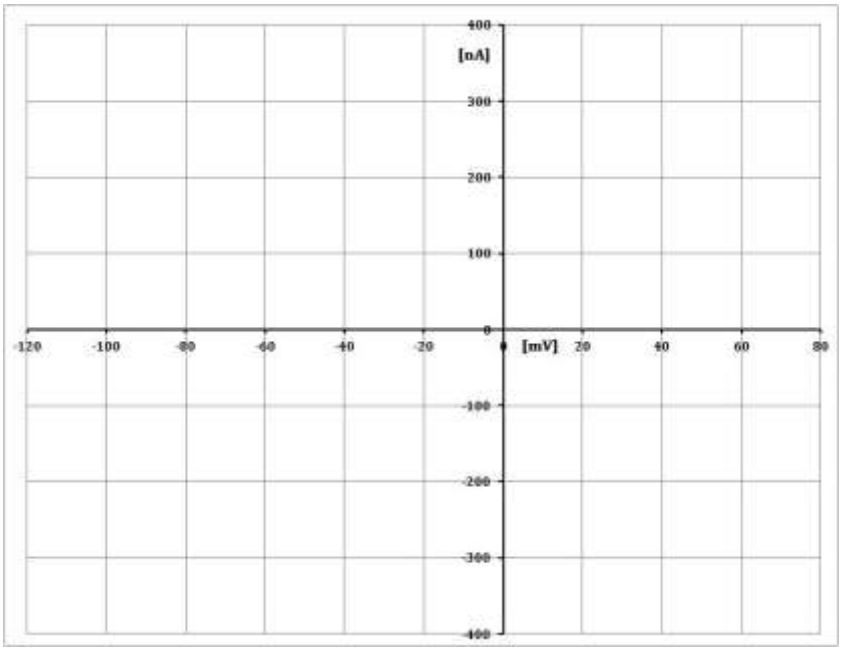
Please apply a command voltage to –30mV and then try to find another command voltage which generates approximately the same maximum Na-current.
Can you explain the different situations?



7. Current-Voltage-Curves, Reversal Potentials, Maximum Conductances

Chart the maximum Na- and K-conductances as a function of different command voltages (table and diagram).
Indicate the Na-reversal potential.
Use your charts to determine the K-reversal potential. Herefore, lengthen the linear part of the I-V-curve until it crosses the x-axis (I=0). For comparison, do the same with the I-V-curve for Na-currents.
Calculate the maximum conductances for voltage dependent Na- and K-currents ($g_{Na,max}$ and $g_{K,max}$) from the linear parts of the I-V-curves.

V_C	I_K	I_{Na}



Reversal potentials: V_K = _____ V_{Na} = _____

Maximum Conductances: $g_{max} = \Delta I / \Delta U$

$g_{Na,max}$ = _____ $g_{K,max}$ = _____

Questions:

1) Can you explain the linear I-V relations and the deviations thereof?

2) Which reversal potential would you expect when you block both the specific Na- and K-channels but open an unspecific cation channel I_C which is equally permeable to Na- and K- ions?
V_C = …….

3) Can you interpret the situation when you find a reversal potential of about 0mV (as it is the case for the Acetycholin gated channel at the neuromuscular junction)?
Is this channel better conducting Na- or K-ions? What is the ratio of the Na and K conductances?

8. Voltage Dependent Activation and Inactivation Curves

8.1 Chart the **activation curves** as a function of different command voltages calculating the I/I_{max}-quotient from your diagram in task #7 (table and diagram)

8.2 Determine the **half-activation potential** (Vh) and the **slope of activation** (s) and try to construct the best fitting Boltzmann-Function a = 1 / (1 + exp(-s*(V-Vh))). Compare your curves with those from the Neuron Editor

V_C [mV]	n = I_K/I_{Kmax}	m = I_{Na}/I_{Namax}

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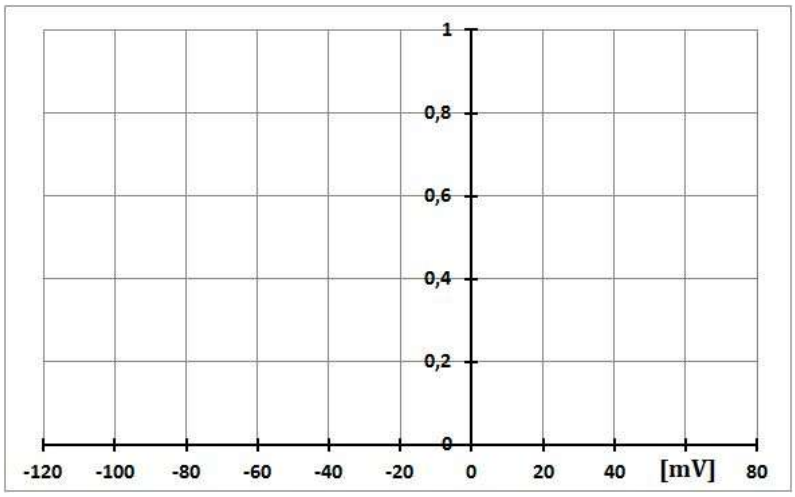
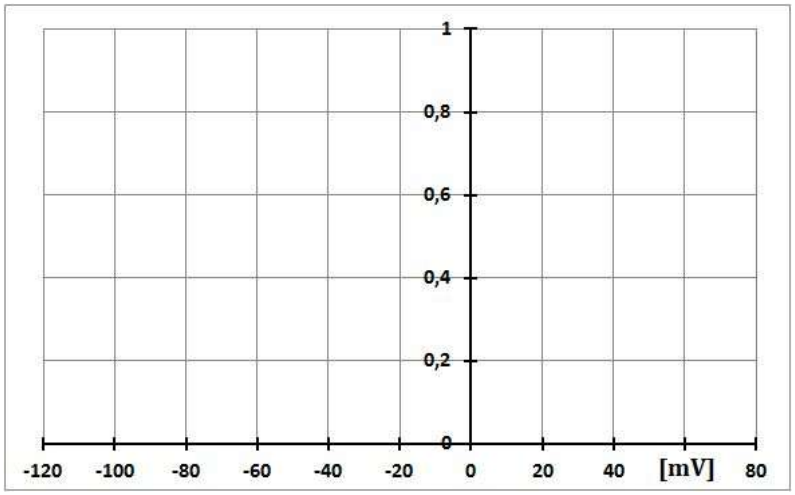




Half-activation potentials:

V_{hK} =

V_{hNa} =

Slopes of activation:
s = ln(1/*a* – 1) / (Vh-V)

s_{Na} =

s_K =

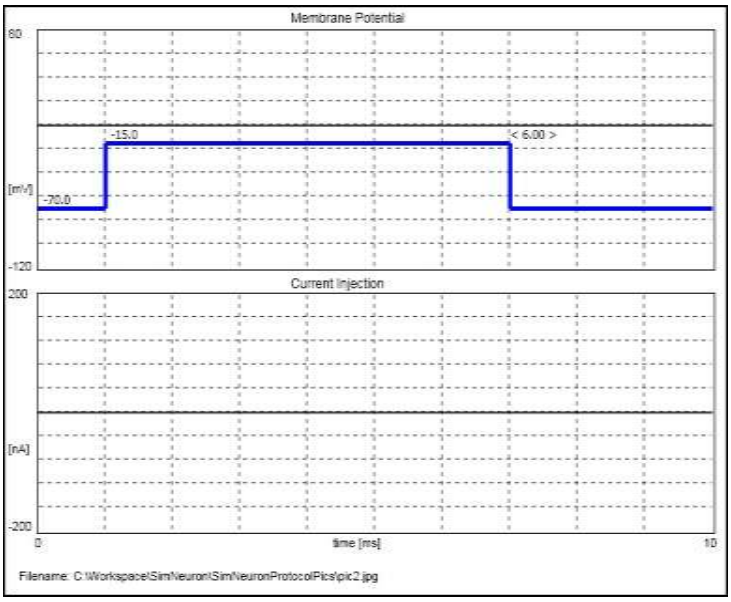
8.3 Chart the inactivation curve a as a function of different hold voltages (recordings and table)

8.4 Determine the half-inactivation potential V_{ihNa} and the slope of inactivation s_{iNa}

8.5 Construct the Boltzmann-Function of inactivation $h = 1 - 1 / (1 + \exp(-s_{iNa}*(V-V_{ihNa})))$.

Table

V_C [mV]	I_{Na} [nA]	h



Half-inactivation potential:

V_{hiNa} =

Slope of inactivation:

s_{iNa} =