Introductory Neuroscience and Neuro-Instrumentation
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Lecture 48
Demonstration: Resting Membrane Potential

Introductory Neuroscience and Neuro-Instrumentation. This is the demo of the resting membrane potential.

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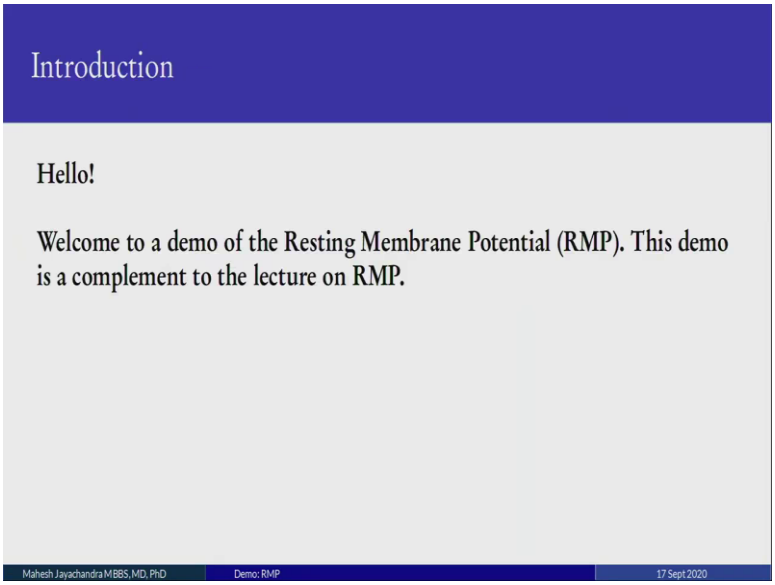

Hello and welcome to our demo of the resting membrane potential. This demo is a complement to the lecture on the resting membrane potential in the Introductory Neuroscience and Neuro-Instrumentation course.

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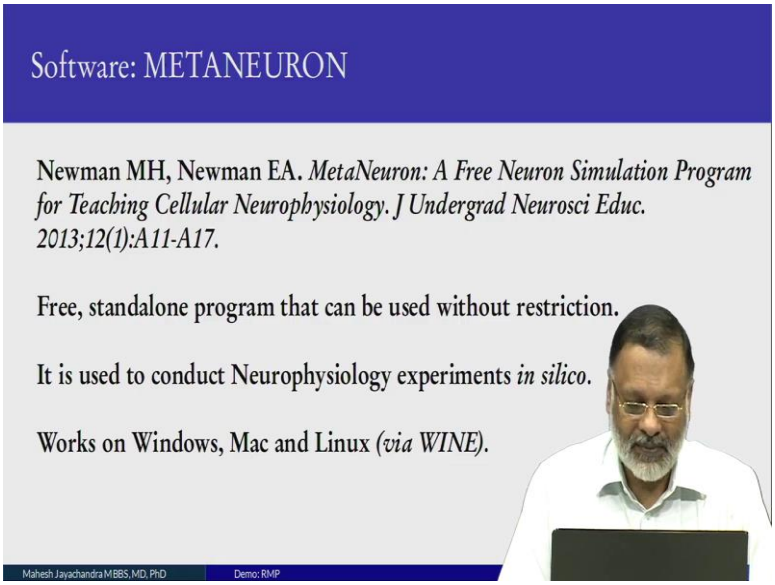

So, before I start, some general remarks, this stuff, the resting membrane potential and the action potential, these are counter intuitive and usually students find it difficult to understand the concepts just by reading. So, to aid students, many many groups, professors, scientist have made programs which work on the Window, Mac or Linux platforms and which show the resting membrane potential like what happens to the potential when you change the ion, ionic concentrations of potassium, change the ionic concentration of sodium so on and so forth.

These through a lot of experience over last 30-40 years by, in neuroscience programs, I have shown that students tend to pick up these things better and have a better intuitive kind of understanding if they actually see it. Now, it is not possible to show some of these experiments because you know, the action potential experiments use squids and those are difficult to get, you need seawater and so on and so forth.

So, these equations, you know, which govern the resting membrane potential and the action potentials happily enough, they provide a complete description of what we need to know to figure these potentials and these phenomena out. So, that is the basis of this demo. We will be showing some of these potentials using a software called MetaNeuron. A MetaNeuron was created by professors Newman and Newman from the University of Minnesota and it seems to be one of the more easier programs to learn these phenomena.

So, the paper which I refer to you is MetaNeuron, a free neuron simulation program for teaching cellular neurophysiology, in June which is journal of Undergraduate Neuroscience Education, from 2013 and it has been used in many programs satisfactorily and that is why

we chose it. The other thing is it is free and it is a standalone program and can be used without restriction. And it is used to conduct neurophysiology experiments in silico as opposed in vivo. It works on Windows, Mac and Linux, Linux you have to use program called WINE which allows you to run this program.

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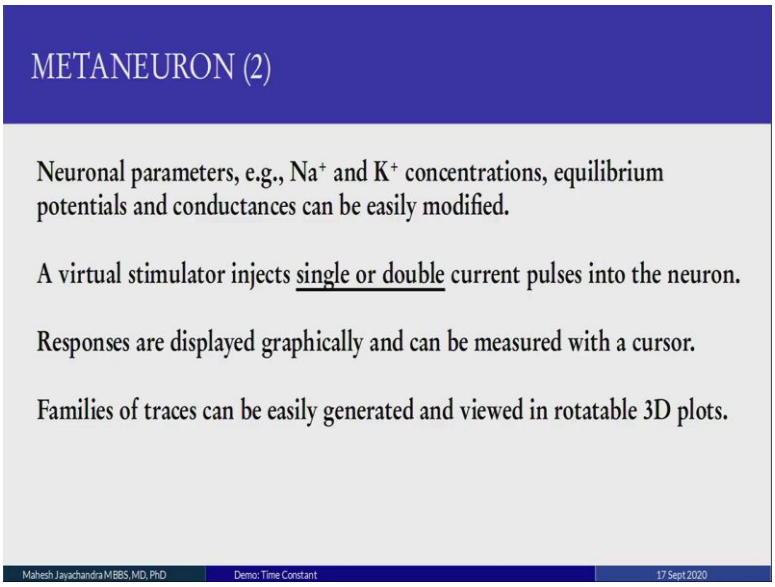

So, the different neuronal parameters that is sodium, potassium concentrations, their equilibrium potentials and conductances can be easily be modified. Also you can inject current, either a single or a double current pulse into the neuron to see its effects on the potentials. And the responses are displayed graphically and can be measured with a cursor. Also families of traces can be easily generated.

Suppose you vary the extracellular sodium between 10 mill molar to 100 millimolar and you increase it in jumps or steps of 10, we can generate a family of traces. So, we can observe what happens at different concentrations. And finally, it can be viewed, these family of curves, traces can be viewed in rotatable 3D plots which helps in understanding it further.

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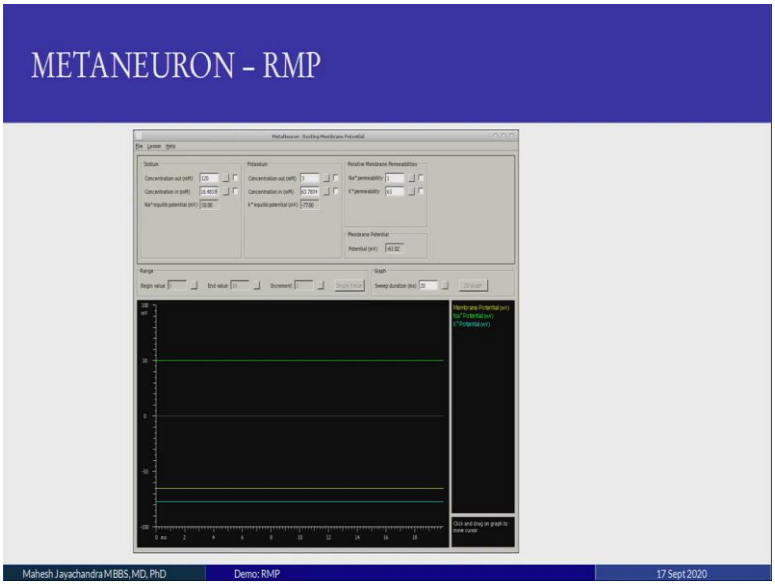

So, I strongly advise you to download this program, as I said it is free, install it on your system, also download the paper and there are a lot of students exercises and if you go through the students exercises, you have a very good understanding of what exactly happens and how the phenomena of the membrane potential and action potential work.

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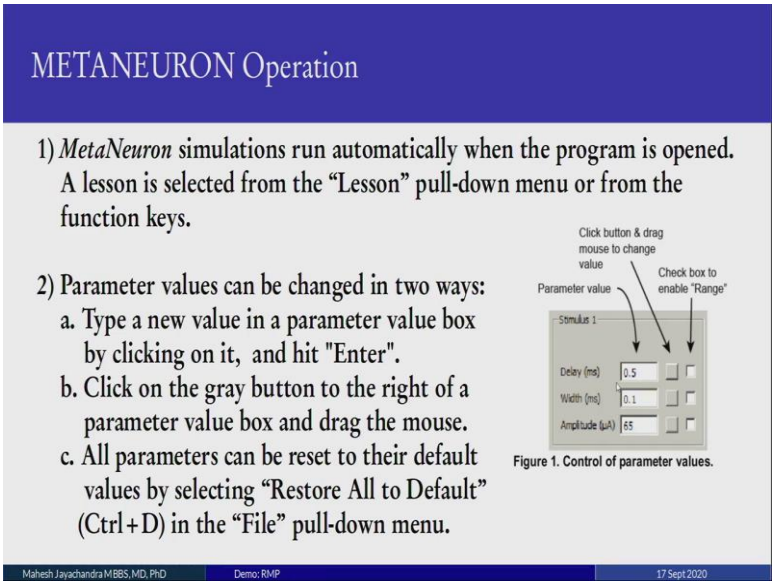

So, a brief intro to the operation of the program. These simulations run automatically when the program is open and you select a lesson from the "Lesson" pull-down menu or from the function keys. So, the lessons deal with the membrane potentials, resting membrane potential, the action potential, voltage clamps, synaptic potentials, so on, the length constant and the time constant.

So, the parameter values can be changed in two ways. You can either type a new value in the parameter value box and by clicking on it and hit enter or you can click on the grey button to the right of the parameter value box and drag the mouse. If you drag it in the right side, it increases, if you drag it to the left side, it decreases. And finally, all the parameters can be reset to the default values by selecting “Restore All to Default” (control D) in the “File” pull-down menu. And this is for your one shows how you can control the parameter values.

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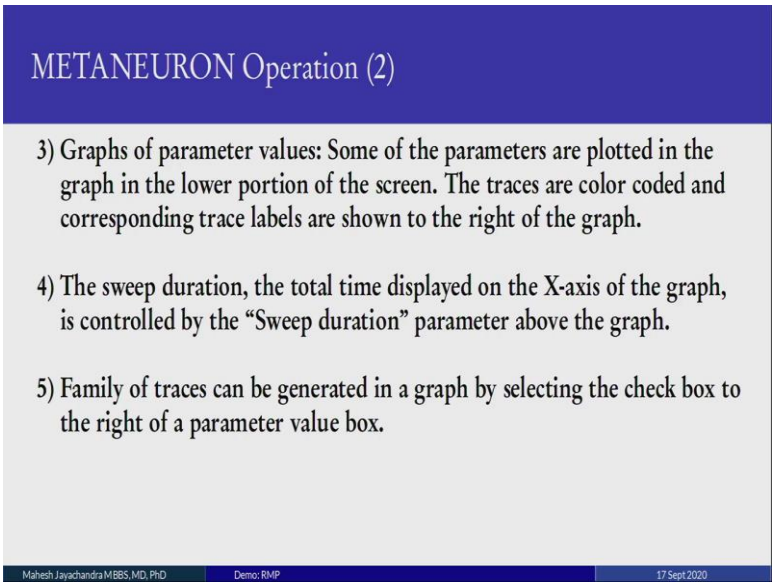

Then the graphs of parameter values: some of the parameters are plotted in a graph on the lower part of the screen, the traces are colour coded and corresponding trace labels are shown to the right of the graph. Also the sweep duration, that the total time displayed on the X-axis is controlled by “Sweep duration” parameter just above the graph. And as mentioned earlier, the family of traces can be generated in a graph by selecting the check box to the right of the parameter value box.

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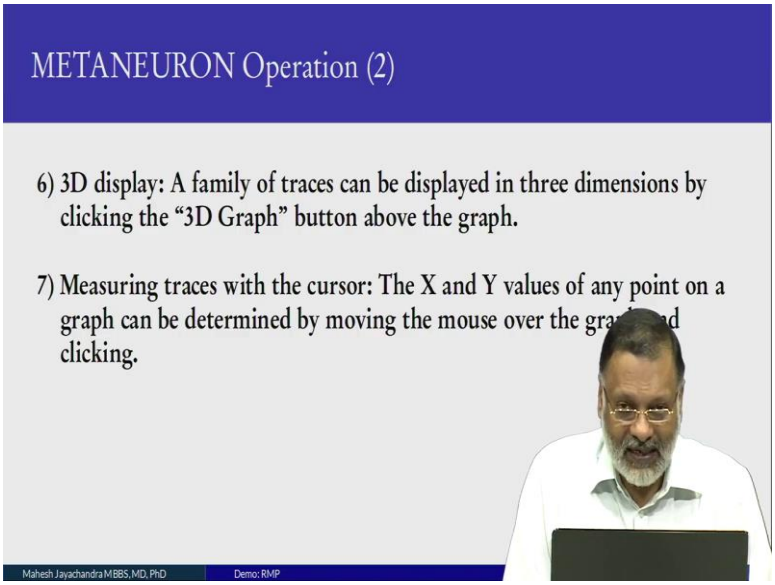

A 3D display: A family of traces can be displayed in 3 dimensions by clicking the 3D graph above the plots. And finally, if you want to measure the trace with the cursor, the X and Y values of any point on the graph can be determined by moving the mouse over the graph and clicking.

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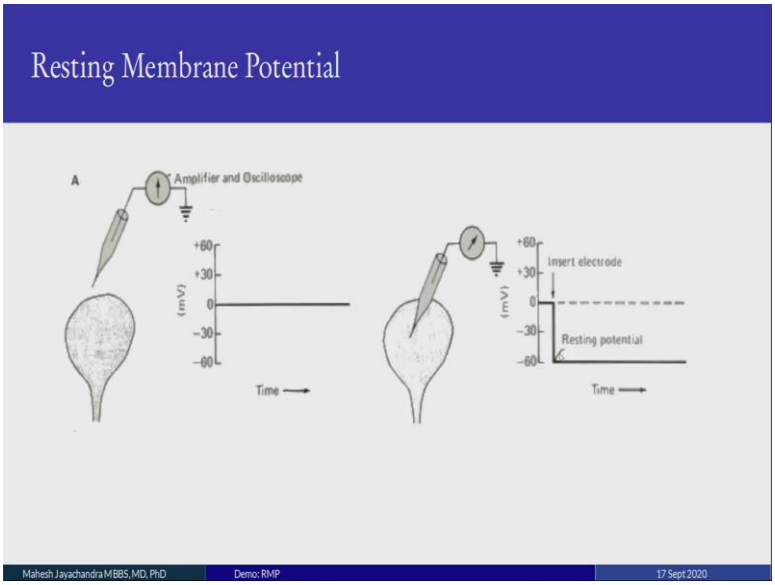

So, the resting membrane potential, how do you measure the resting membrane potential? So, you need an intercellular electrode and on the left you see the intercellular electrode outside the cell and you do not have any potential because it is outside, so it is 0. It is 0. On the right, the intercellular electrode has been inserted inside the cell and we are recording from inside

the cell to the ground and soon as you insert the electrode, the potential goes to minus 60 millivolts and that is the resting membrane potential.

If you take it out, it will again come back to 0. Now, we are going to go through the demo to see how the resting membrane potential arises.

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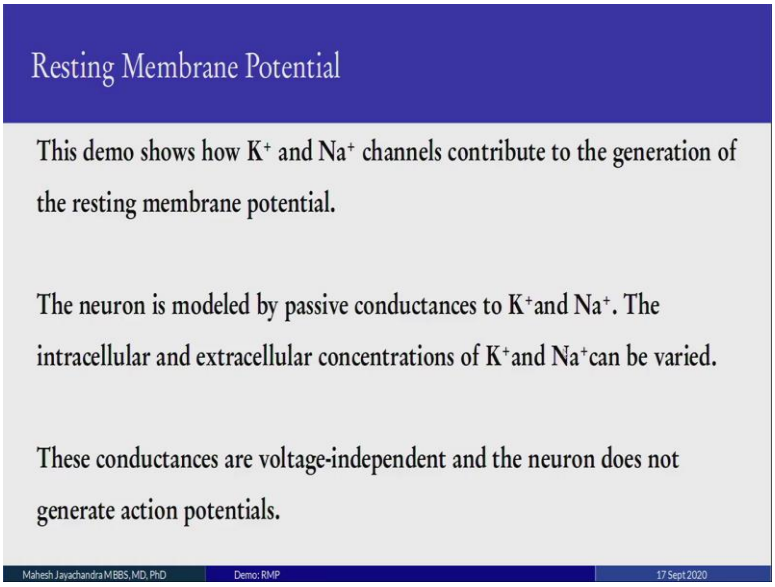

So, basically, why potassium and the sodium channels contribute to the rest generation of the resting membrane potential. The neuron is model by passive conductances so, of potassium and sodium and the intercellular and extracellular concentrations of potassium and sodium can be varied. So, these conductances remember our voltage-independent and the neuron does not generate action potentials in this model.

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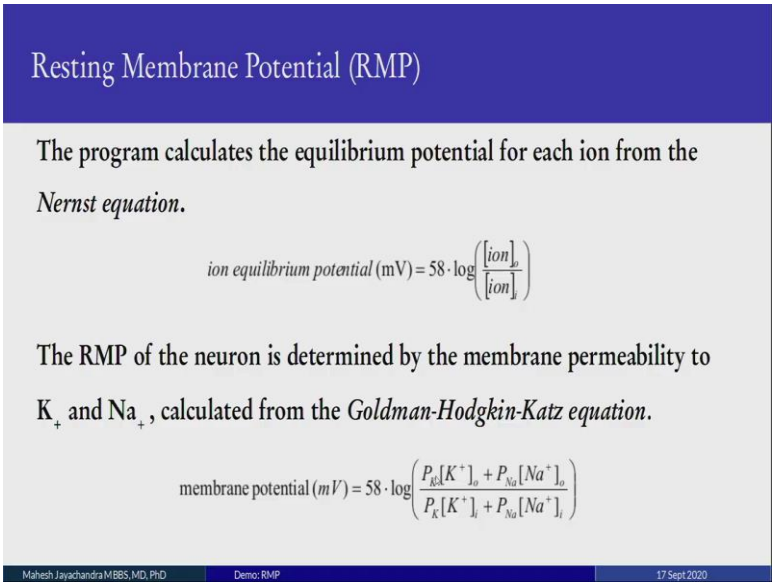

So, just to remind you, suppose you just have one ion, the resting equilibrium potential for that particular ion is given by the Nernst equation, Rt by Gf, natural log of extracellular concentration divided by intercellular concentration. So, that can be at the usual temperature use, all those coefficients can be reduced to 58, then use log to the base 10 and then it is an extracellular ion concentration, the square brackets indicate molar concentrations versus this intercellular.

Now, this is just for a single ion base sodium to be potassium. The resting membrane potential neuron, however, is determined by an interplay of the other ions. And that is an extension of the Nernst equation and is calculated from the Goldman-Hodgkin-Katz equation, the GHK constant field equation if you remember and here is a simplified version of the GHK equation where we just considering the main ions contributing to the resting membrane potential which is potassium and sodium. And P stands for permeability, how much the ion can go through the membrane, cell membrane.

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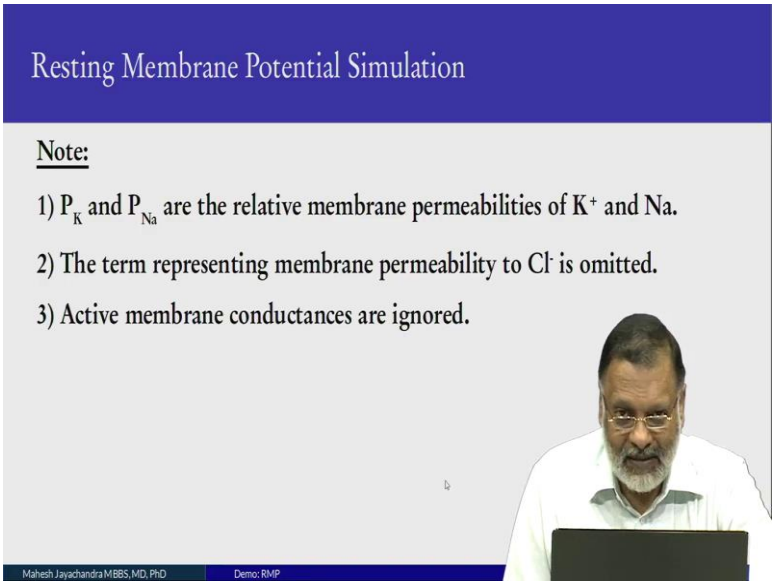

And one note, as I said P, P k and P Na are the electrode membrane permeabilities of potassium and sodium. And usually chloride is omitted because it is freely permeable at its equilibrium potential and it is close to the potassium equilibrium potential and the potassium equilibrium potential is a one that contributes majorly to the resting membrane potential. So, ignore chloride and also we ignore active membrane conductances which cause action potentials, we are just looking at the passive membrane conductances.

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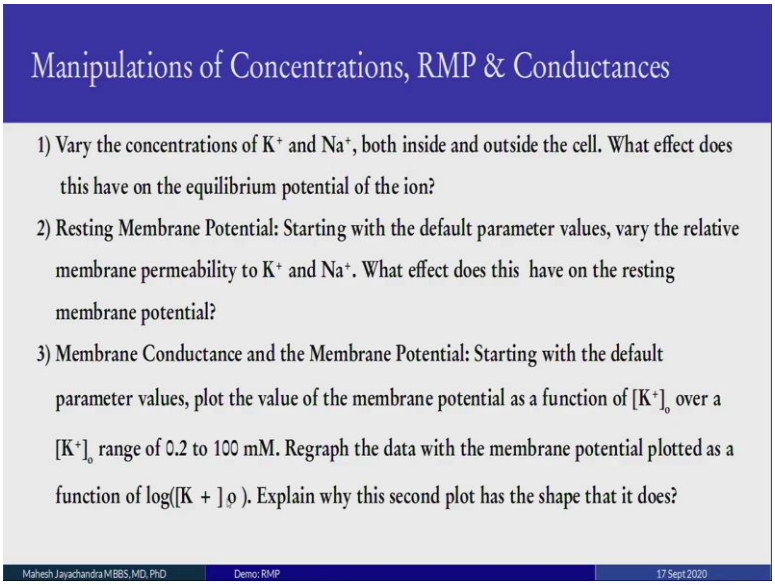

So, some of the different manipulations you can do in the program which I will come to in a minute is vary the concentrations of potassium and sodium, both inside and outside the cell. And you can check it to see what effect this has on the equilibrium potential of each ion. Then also the resting membrane potential itself starting with the default parameters, we can vary the membrane permeability, how much of potassium or sodium is allowed to go through the membrane and see what effects this has on the resting membrane potential.

We can also look at the membrane conductance and membrane potential, we start with the default parameter values and we can plot the value of the membrane potential as a function of extracellular potassium over a range. For example, 0.2 to 100 millimolar and we can re-graph the data with the membrane potential plotted as a function of log of potassium outside and you can try and figure out why the second plot has the shape it does.

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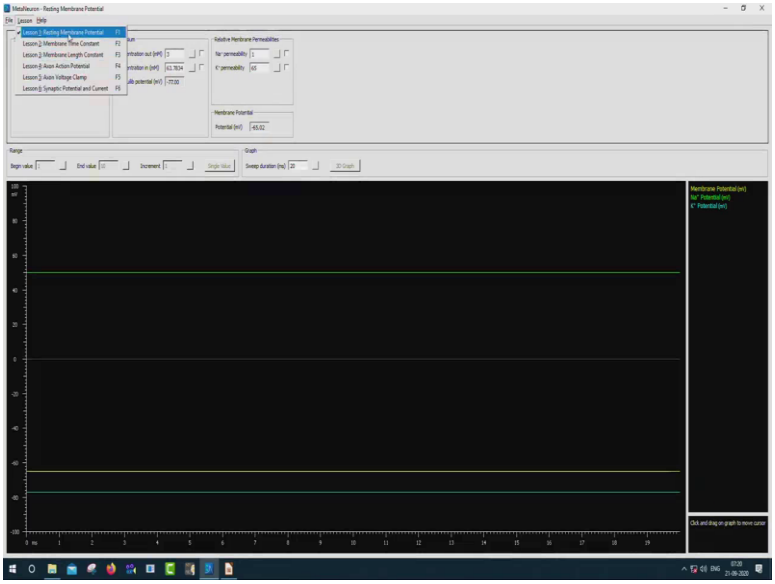

So, now let us go to the demo. So, this is MetaNeuron, and the resting membrane potential demo. So, just briefly, there are 6 lessons, the resting membrane potential, membrane time constant tau, the membrane length constant lambda, then the action potential, then the action voltage clamp and finally synaptic potentials and current. So, we will start with the resting membrane potential. So, the left, you see sodium concentrations outside the cell, so this is 120, concentration inside the cell is 16.48 and given these two, from the Nernst equation, we get a sodium equilibration potential of 50.

So, below are the plots and the membrane potential is yellow, right here. The sodium equilibration potential is 50, right on top over here and the potassium, equilibrium potential, given the values above is minus 77. So, potassium is on next to the sodium and again the concentrations inside and the concentrations outside, so outside is 3, inside is some about 63.7 and next to it is the relative membrane permeabilities, the sodium permeability is 1 and the potassium permeability is kept at 65.

And with all these values you get the membrane potential at minus 65, that is over here. So, we will start and the sweep, whereas the sweep is not really important in this particular demo, but the sweep over here is 20 milliseconds.

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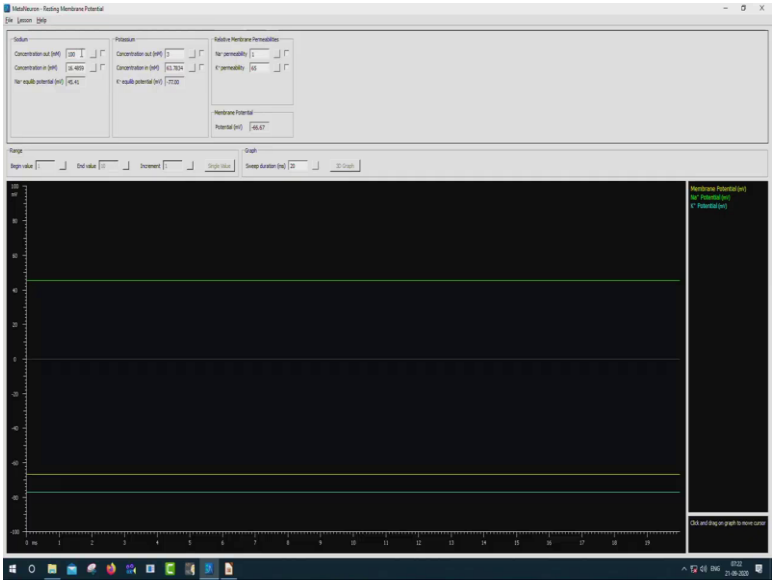

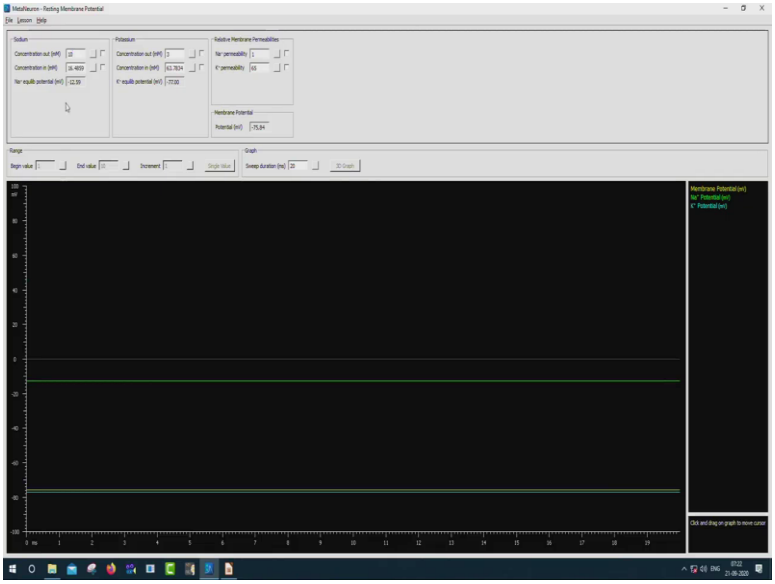

So, we will start by varying the sodium concentration. So, I am changing the sodium concentration to a 100 and press enter and you see it comes down. So, outside remember is seawater, so NaCl. So, remember if sodium is not present outside, you will not have action potentials, so you see how the membrane potential goes down when we decrease the sodium concentration, so I make it 10.

So, not only the sodium here with a major decrease in extracellular sodium, the sodium potential goes down, the sodium equilibration potential and so does the membrane potential. It is gone, it is really close to the potassium equilibration potential.

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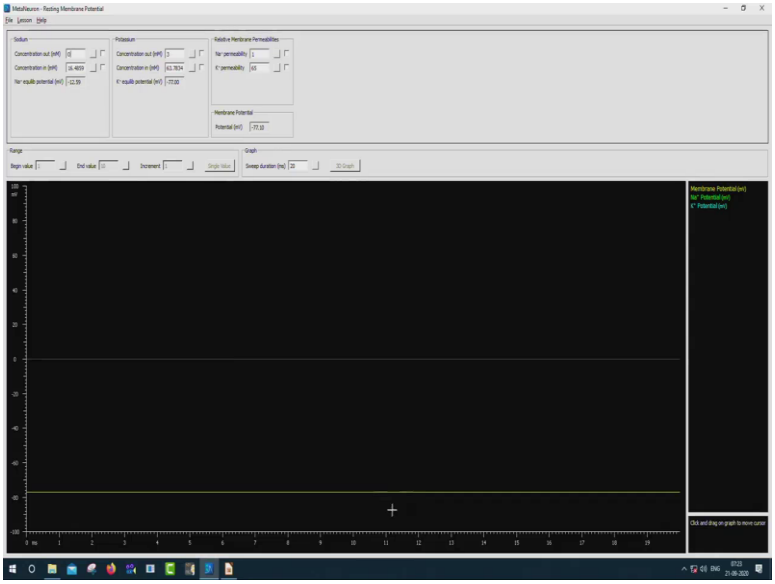

And what happens when I make it 0, it is gone. So, here since there is no sodium, there is no sodium outside, sodium inside does not matter. Sodium outside is 0, the equilibration potential, that is resting membrane potential is equal to the potassium equilibration potential because that is the only ion in play right now. So, we go back and restore all the values to the default and we get back the standard sodium potential, the equilibration potential, and the potassium potential.

So, let us do the opposite. What happens when it mess with the or change the potassium concentration? So, the potassium concentration inside is what matter, so it is about 64.

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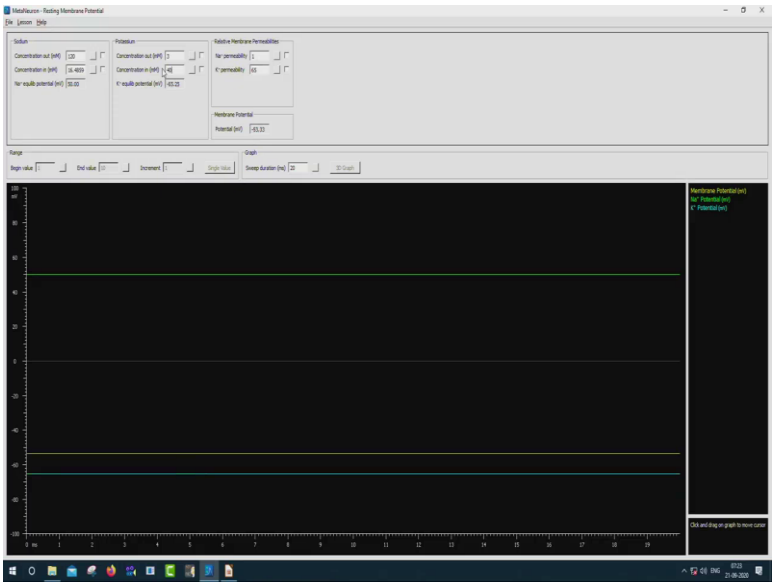

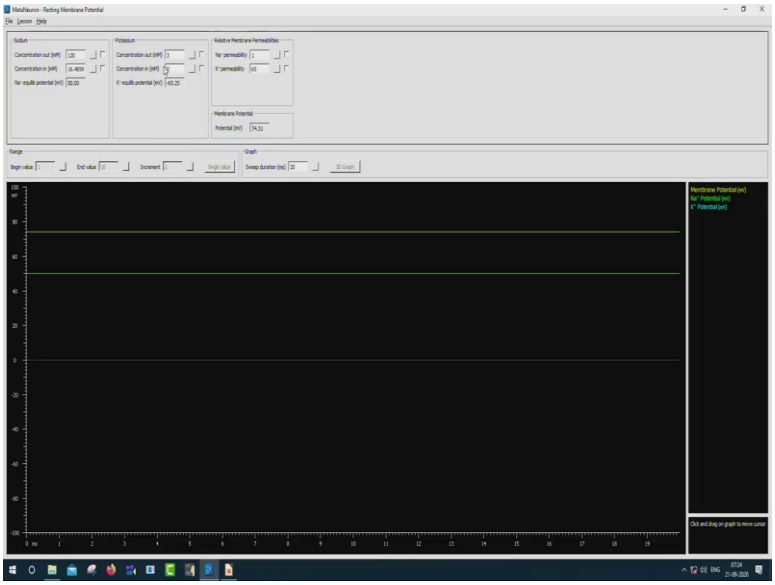

So, I am going to make it 40. So, not only does it come up, but the equilibration potential also changes, it goes up and I will make it 0. So, when we make it 0, basically there is no ionic force due to the potassium and the membrane potential goes way up. There is only sodium acting over here. So, coming back to let us restore to default so, what happens when we change the permeability?

Remember, at rest, at the resting membrane potential, there is no sodium influx and into the cell, whatever little there is, it is reverse by the sodium potassium ATPs. And it is very low compared to the potassium conductance.

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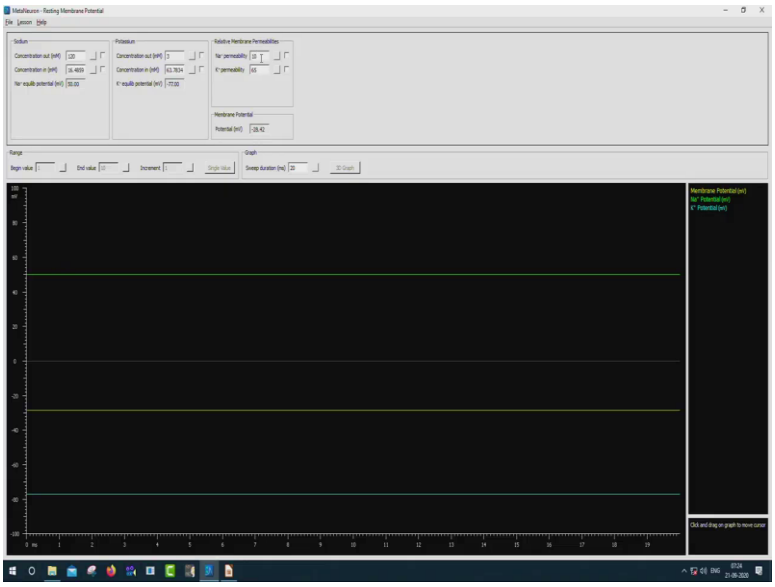

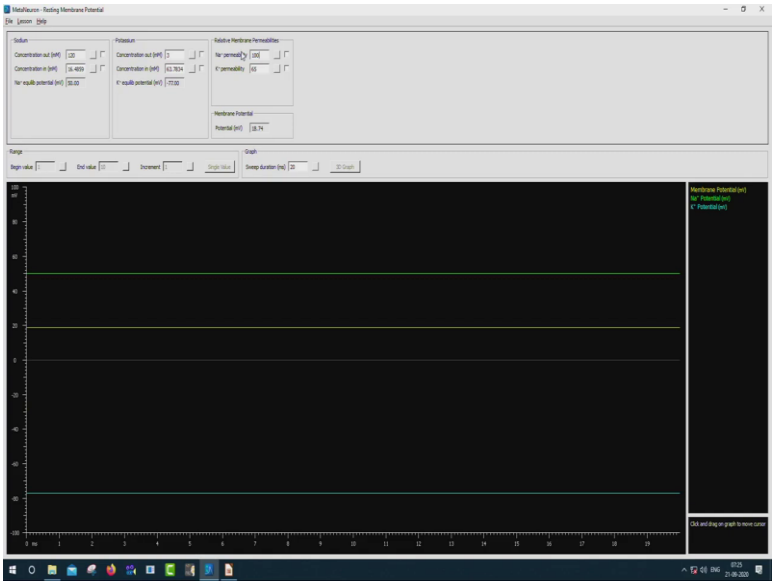

So, if I changed it to 10, notice the change in the membrane potential. If I change it to a 100, so it is much more than the potassium, again it goes up. So, playing this, these different values, playing with the sodium concentration, the potassium concentration and the relative membrane permeabilities, you get an idea of the behaviour of the resting membrane potential.

When you change the concentrations, when you change the permeabilities, etcetera. Now, this is a little contour intuitive because the equation is not a linear equation and we find that students find it much easier to understand the RMP when they change these values. So, I leave the rest of the manipulations to you and as I said this is just a demo and we will not be asking questions on the demo.

But if you want to get a better understanding of how exactly the resting membrane potential works, I strongly advise you, suggest you to install the this program, MetaNeuron on your system, and also read the paper and it also has a manual for students. So, you systematically go through the different exercises and that way you know you can work at a phase at home and get an idea of the RMP.