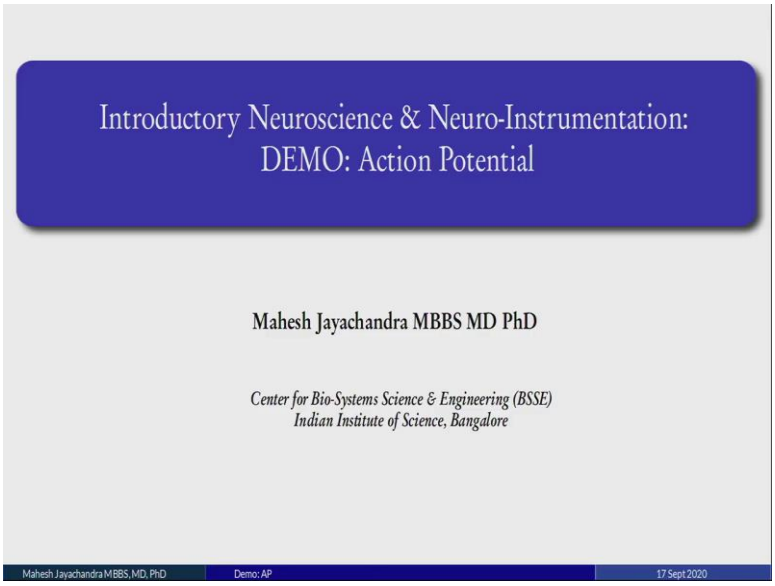
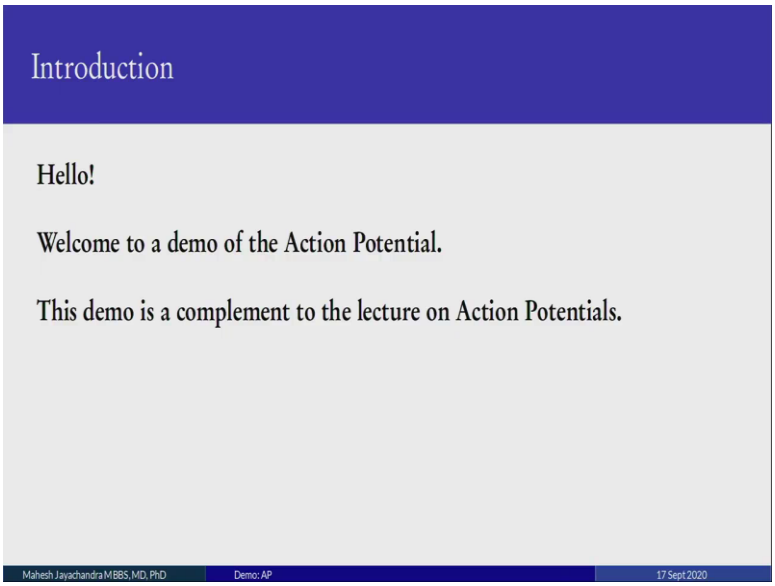
Introductory Neuroscience & Neuro-Instrumentation
Professor Mahesh Jayachandra MBBS MD PhD
Center for Bio-Systems Science and Engineering
Indian Institute of Science, Bangalore
Lecture No. 55
Demonstration: Action Potential

(Refer Slide Time: 0:23)



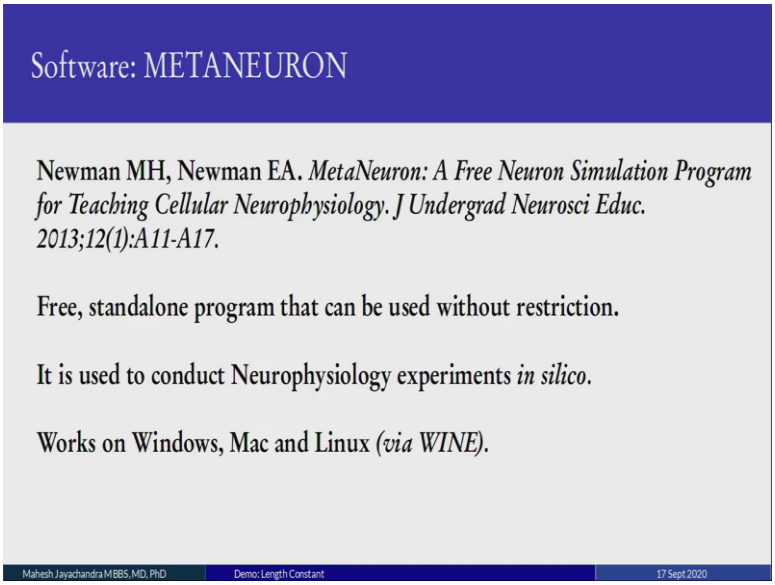
Introductory Neuroscience and Neuro-Instrumentation and this is a demo on the action potential.

(Refer Slide Time: 0:24)



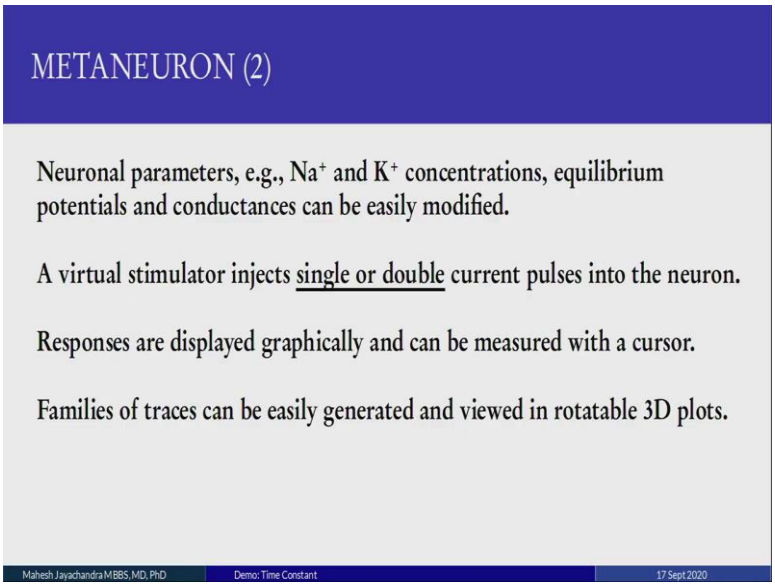
And this is a complement to the lecture on Action Potentials.

(Refer Slide Time: 0:29)



So, again we are using the same MetaNeuron program made by Doctors Newman and Newman from the University of Minnesota. It is a free neuron simulation program for teaching Cellular Neurophysiology and it was published in the journal of Undergrad Neurosci Education, June 2013. So, it is a free standalone program that can be used without restriction and it is used to conduct neurophysiology experiments in silico. It works on Windows, Mac and Linux, with Linux it works via WINE.

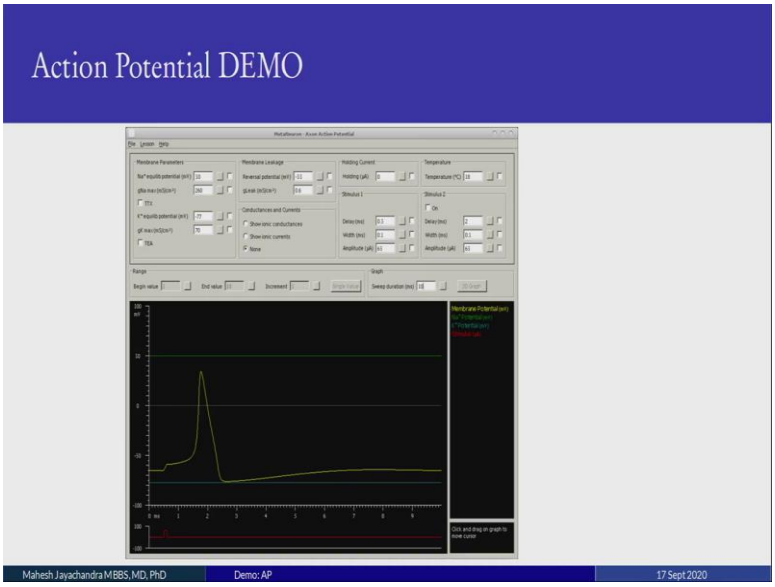
(Refer Slide Time: 0:59)



And as mentioned earlier, different neuronal parameters like potassium, sodium concentrations, equilibrium potential and conductances can be easily modified. And a virtual stimulator injects single or double current pulses into the neuron. So, responses are displayed

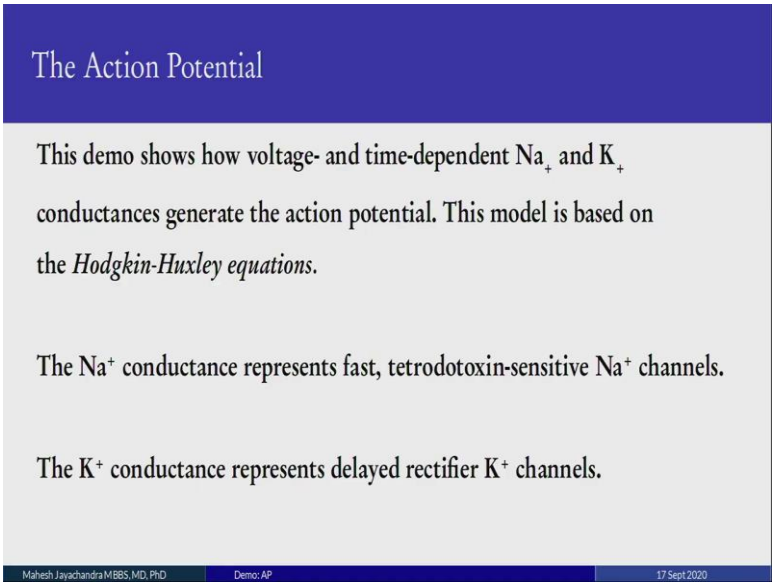
graphically and can be measured with a cursor. And you have family of traces that can be easily generated and viewed in rotatable 3D plots.

(Refer Slide Time: 1:25)



So, this is actually the easy one because the earlier demos were on the resting membrane potential, tau and lambda, these are kind of esoteric concepts. But the action potential, the good all action potential is something which is everyone knows and this is how axons work. So, this is far easier to understand.

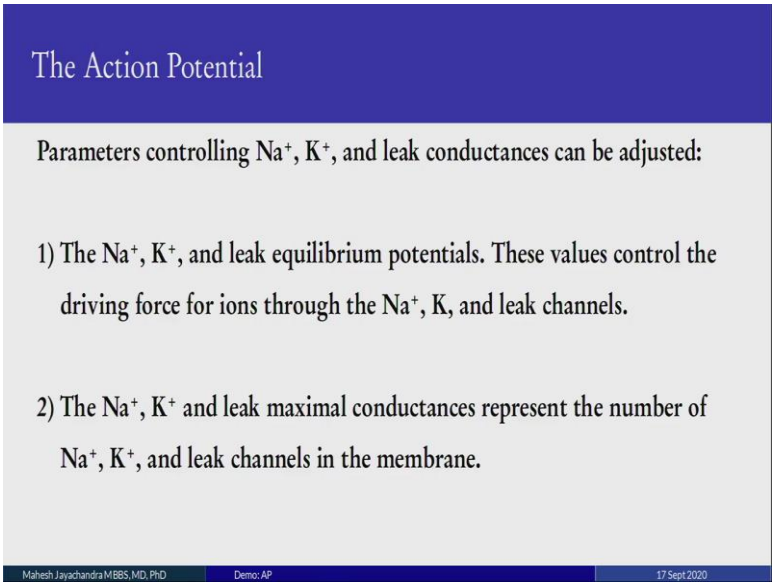
(Refer Slide Time: 1:49)



So, this demo shows how voltage and time dependent sodium and potassium conductances generate the action potential. This model is based on the Hodgkin-Huxley equations and for which they got the Noble prize. And the sodium conductance represents fast TTX,

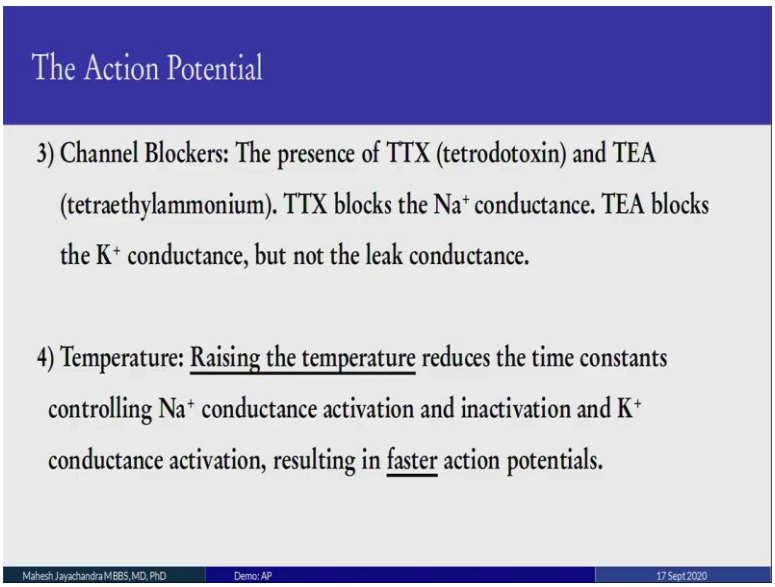
Tetrodotoxin sensitive sodium channels. The potassium conductance represents the delayed rectifier or the delayed rectifier potassium channels.

(Refer Slide Time: 2:16)



So, all the parameters controlling the sodium, the potassium and leak conductances can be adjusted and we can adjust the equilibration potentials of the respective ions. So, these values controls the driving force of ions through the respective channels. And the sodium, potassium and leak conductances, the maximum conductances represent the actual sodium, potassium and leak channels in the membrane. So, that is very interesting that you can have a physical quantity like a conductance representing the number of ionic channels studded in the phospholipid cell membrane.

(Refer Slide Time: 2:55)

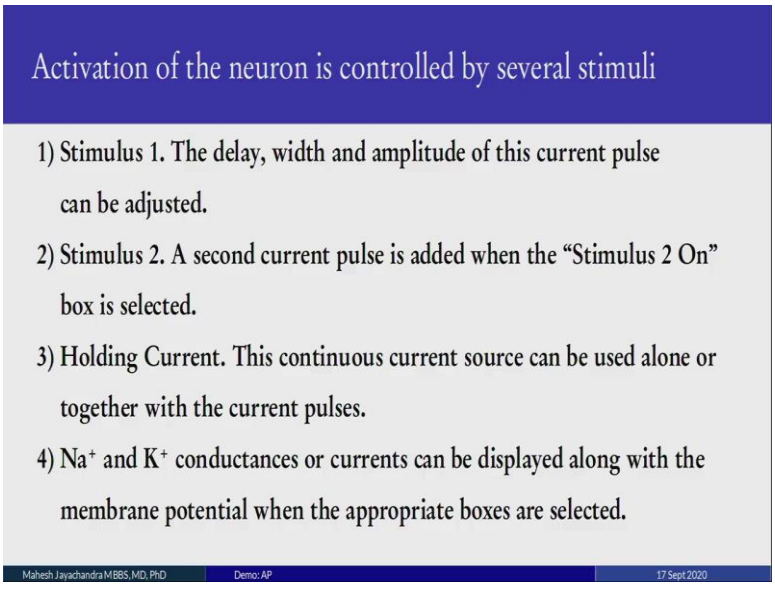


Then we can use channel blockers. So, the presence of TTX and tetraethlyammonium can selectively block channels, the sodium channel is exquisitely sensitive to TTX and when you use TTX, all sodium currents are blocked. Similarly, the potassium channel is sensitive to TEA, tetraethlyammonium and when you use TEA that blocks the potassium channels. However, it does not block the leak conductance which still remains.

Another important parameter is temperature. When you raise the temperature, you reduce the time constants controlling the sodium conductance activation and inactivation and the potassium conductance activation, resulting in faster action potential. Now, you may say why is this important? This is important because in a condition like multiple Sclerosis, you have conductance failure because the myelin if you remember, the axon and the myelin which is the insulation layer of the axon that degrades because of an autoimmune pathology in the body.

So, the thresholds go down, the conduction goes down. So, just by cooling the body or cooling the axon, you can have, it reaches threshold and you have some amelioration of the symptoms. So, here you can actually see it when you change the temperature, how the time constants also change.

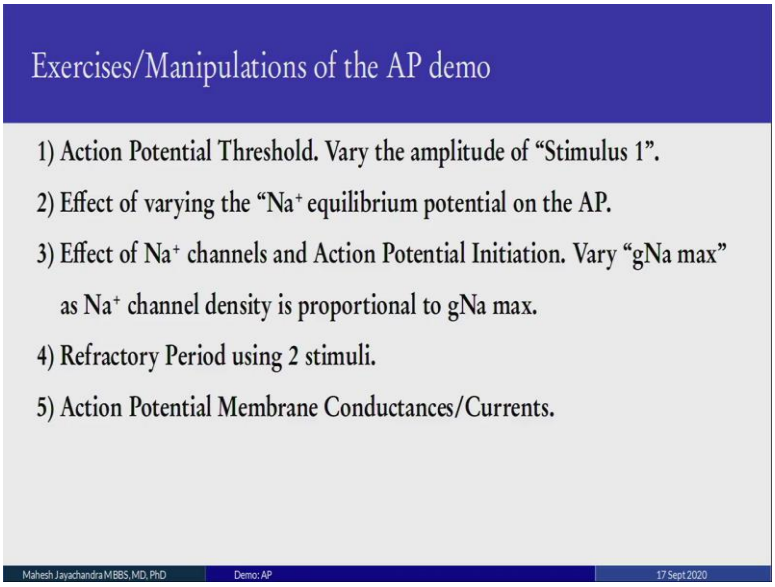
(Refer Slide Time: 4:26)



So, the activation of a neuron, nowhere you have the active conductances which were not there in the earlier three tutorials. So, the activation of the neuron is controlled by several stimuli. Stimulus 1, the delay, width and amplitude of this current pulse can be adjusted and likewise, stimulus 2, when it is on, then you can use holding current, this is a continuous current source, it can be used alone or together with the current pulses. And the conductances

of the sodium and the potassium can be displayed along with the membrane potential when you choose the appropriate boxes.

(Refer Slide Time: 5:01)

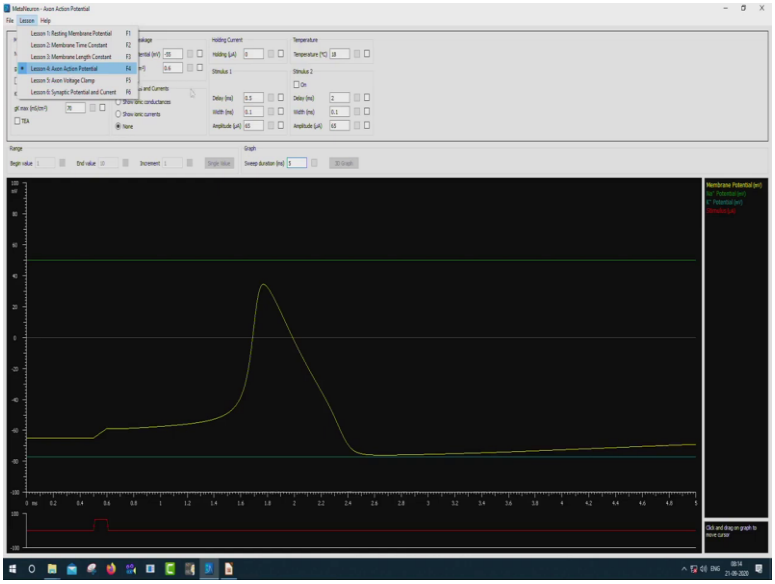


So, some of the different exercises you can do is you can vary the action potential threshold by varying the amplitude of the stimulus. So, you can vary, see the effect of varying the sodium equilibrium potential on the action potential. Then you can see the effect of sodium channels and action potential initiation by varying gNa max, with g stands for the conductance of the sodium through the membrane and it actually, gNa max reflects on the sodium channel density because the sodium channel density is proportional to its conductance, its maximum conductance.

You can also look at the refractory period, if you remember, refractory period, an action potential once a neuron generates an action potential for a couple of milliseconds after you whatever the current you give, it is not going to stimulate another action potential. And then for a little time after that, so that is the absolute refractory period. For a little time after that is the relative refractory period when you can get an action potential but you have to use a huge super threshold current.

And then after that it comes back to normal. So, you can study those phenomena by using 2 stimuli and then also you can see the effect of the action potential membrane conductances and currents on the shape of the response.

(Refer Slide Time: 6:29)



So, this is the MetaNeuron displaying lesson number 4 which is the action potential, not dendrite but axon. So, here unlike as I said, the previous demos you have active voltage conductances and it kind of looks little plump. So, we shall change of the sweep duration. So, that it is more tractable. That is how it is usually displayed. So, here you see the action potential in all its glory. So, just to retreat the red trace is the stimulus in microamps, the membrane potential reaches threshold with this level of stimulation and gives rise to the action potential.

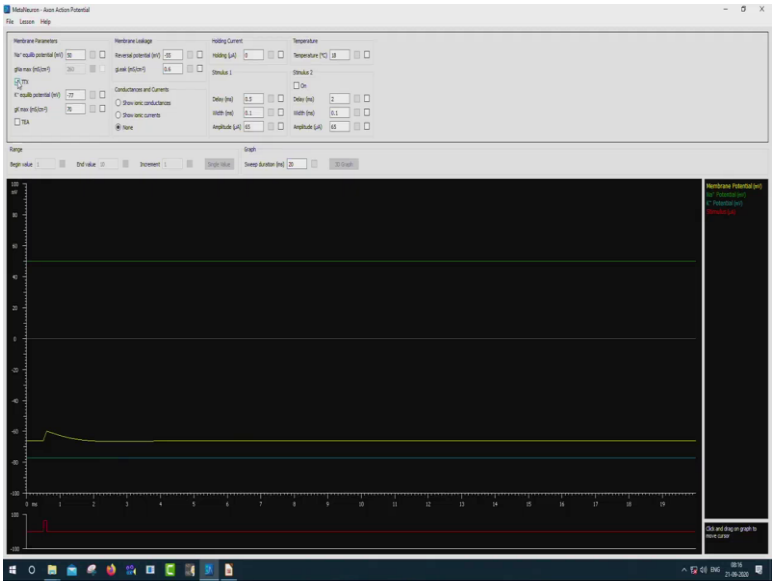
So, this is the upstroke, this is the downstroke and then it goes below its resting membrane potential, so this is the hyper pole, this is depolarisation and this is hyperpolarization and then after hyperpolarization, then it comes back to normal. So, you have membrane parameters

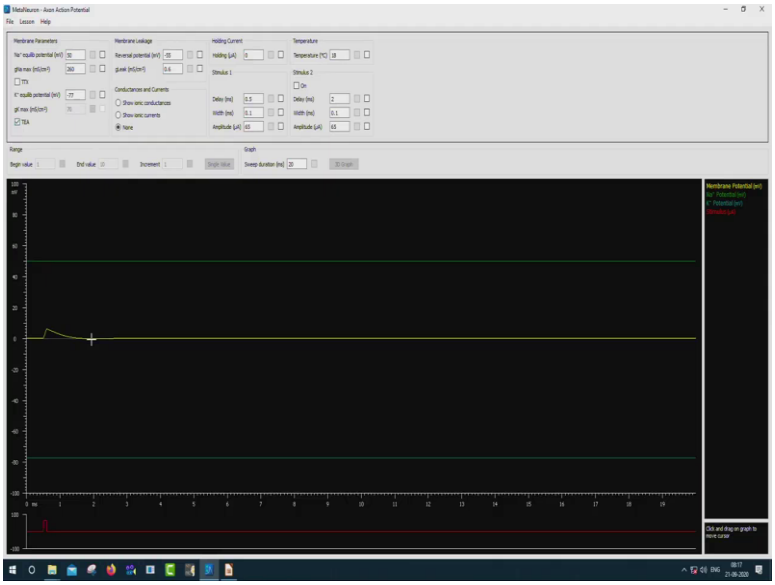
over here. This is the sodium and the most important ion in the resting membrane potential is potassium, but here it is sodium and the sodium equilibration potential is set at 50. So, the conductance g max which reflects the number of sodium channels is kept at 260.

Likewise, the potassium equilibration potential is minus 77 and the g max is 70. And the green trace reflects the sodium equilibration potential at these concentrations, the blue line reflects the potassium equilibration potential again at these concentrations. And these are the reversal potential for the membrane leak, so we can ignore it. And here, right now we can also show the ion conductances and we can show the ionic currents. We are right now doing none of that, we are just showing the membrane potential as it changes.

And holding current, we are not changing. This is stimulus 1, it is on right now and there is a delay of 0.5, so it starts 0.5 milliseconds of from the beginning of the sweep. Its width is 0.1 milliseconds, this is 0.5 milliseconds, this is 0.1 milliseconds, and the amplitude is 65 micron amps.

(Refer Slide Time: 9:05)

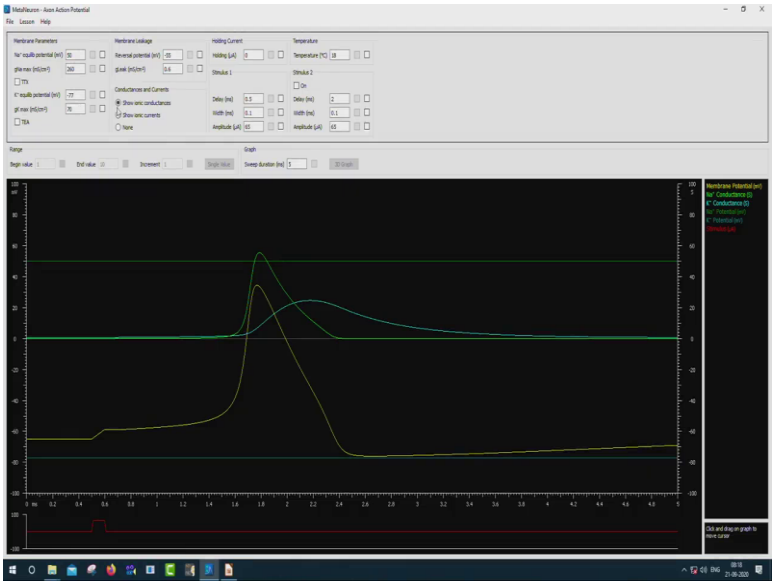




So, let us start with blocking the sodium current. So, hit TTX, that is it, the sodium current is blocked, there is no action potential. I have taken out the TTX blockage and you have the action potential. So, let us block the potassium. So, we hit TEA and again there is no action potential. But the membrane potential has gone up to remember 0. We get it back. So, let us go back to defaults because for the ionic conductances and ionic currents, it is easier to see when it is little expanded.

So, now same, nothing has changed, we have just changed the sweep duration, we have decreased it from 20 to 5 which is a default position. So, let us see what happens to the sodium and potassium ions when we show the ionic conductances.

(Refer Slide Time: 10:00)

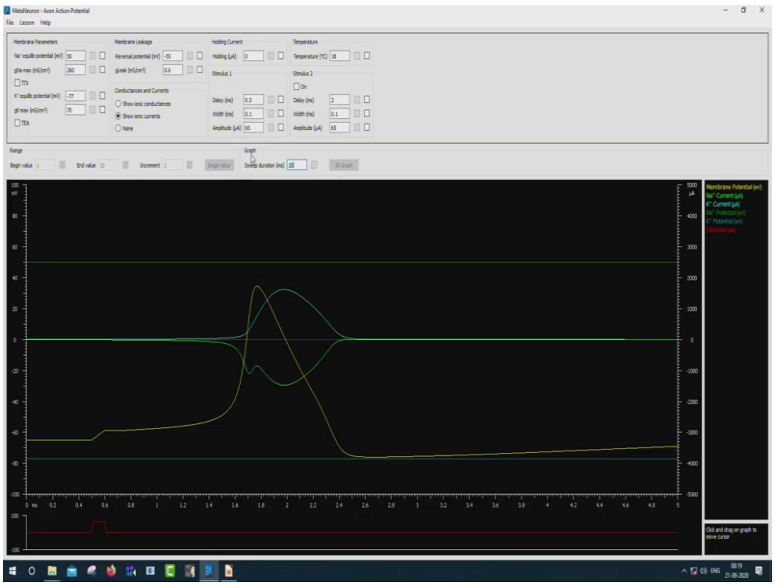


The sodium conductance is this green guy. And the potassium conductance is this glue guy, the trace not guys, trace. So, right at the beginning of the up stroke of the action potential, the conductance for the sodium increases too. So, it goes up and then it inactivates, it comes down. The potassium conductances on the other hand, and that is why it is called a delayed rectifier, it takes off more slowly. It is much slow so it is delayed and then it last for a much longer time right till the end of the action potentials after hyperpolarization and come back to normal.

So, these are the conductances, this is actually innovate, way to think of it is these are channels which are opening, these are sodium channels which are opening, causing the sodium conductance to change, opens rapidly, deactivates, inactivates rapidly, this is the potassium conductance which is opening, opens much slower and does not really deactivate. It keeps going on until the end of the action potential. So, these were the conductances. So, let us look at the currents.

Let us take off the conductances. Again conductances is a actual channels, how they are opening and closing. Now, let us look at the currents which are passing through these conductances.

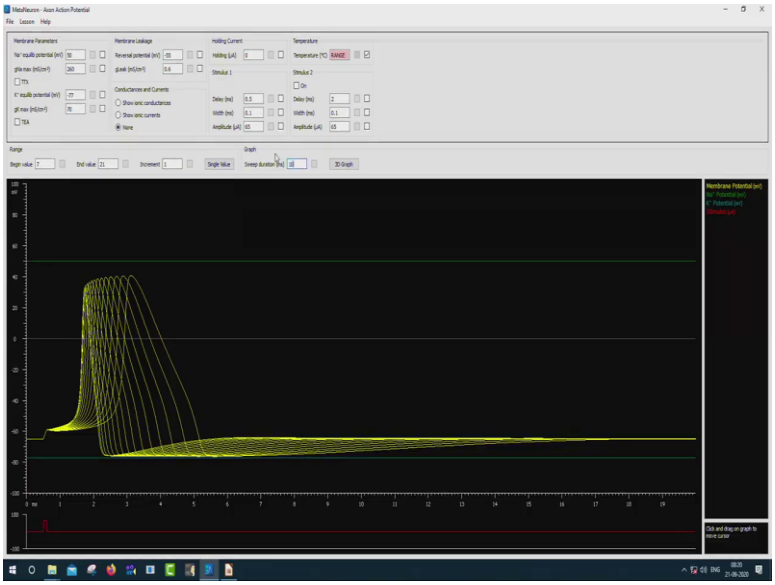
(Refer Slide Time: 11:26)

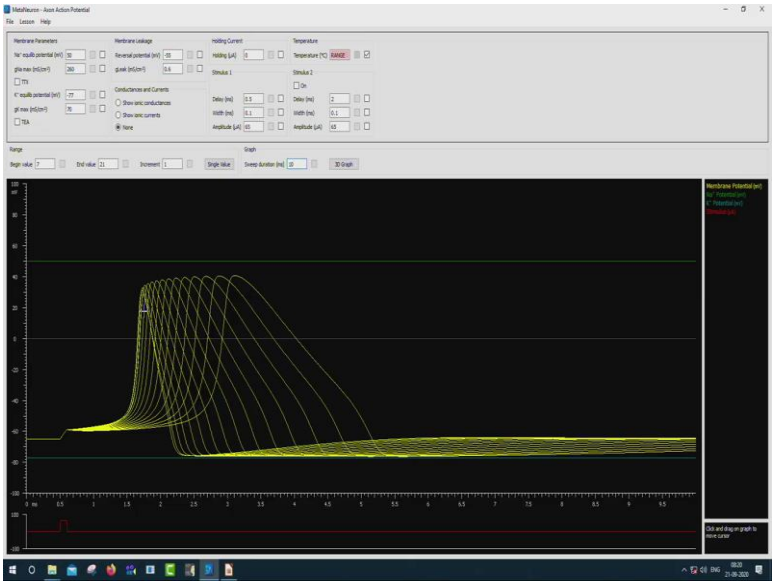


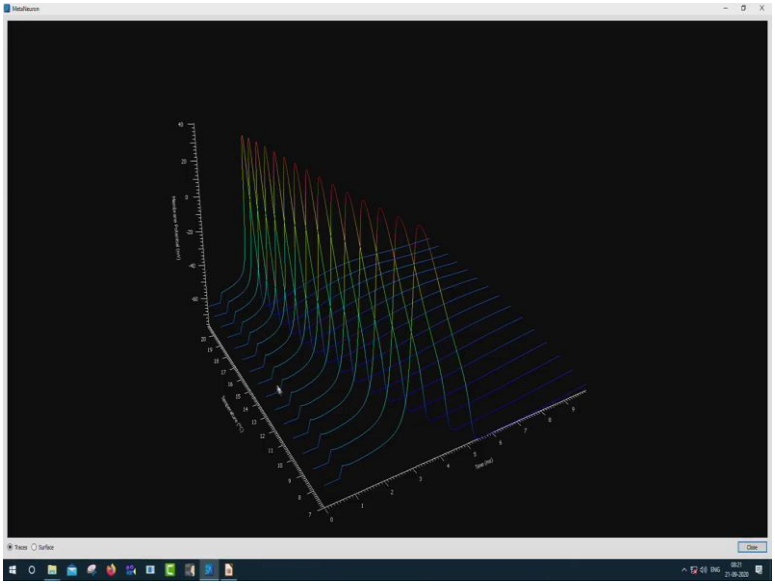
So, this green line is the inward, inward going below inside the axon, inward sodium current and this blue line is the potassium outward current going out. So, this inward current causes the action potential. Remember this is current, while the action potential is voltage. And you have this massive inward current, actually you cannot see it really well over here, I am going to change the duration.

So, you see this massive inward current over here which is the sodium current and you also have a potassium currents starting a little later and so the combination of these two currents, their interplay gives rise to the action potential. So, let us go back to normal. So, what happens when you change the temperature? So, let us look at a range.

(Refer Slide Time: 12:24)



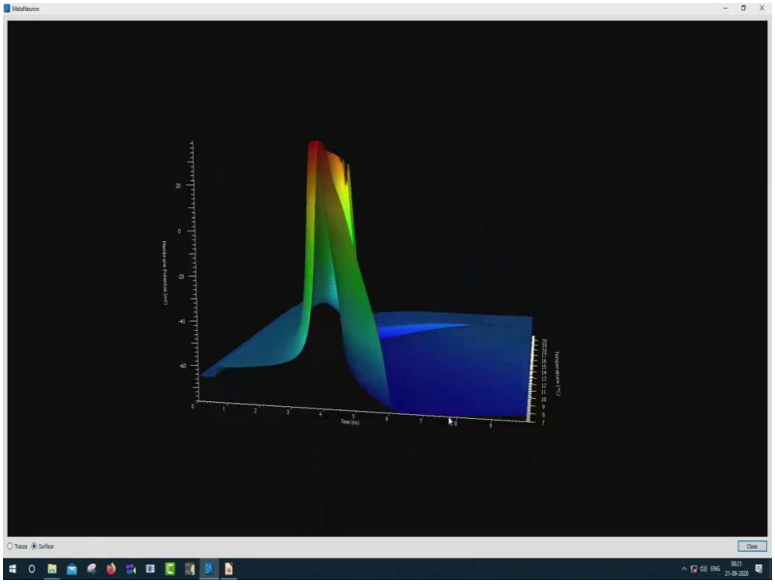


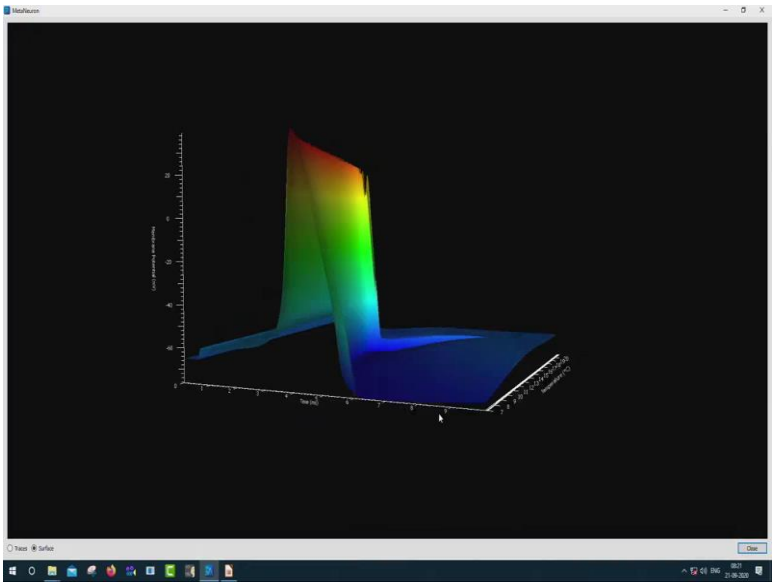


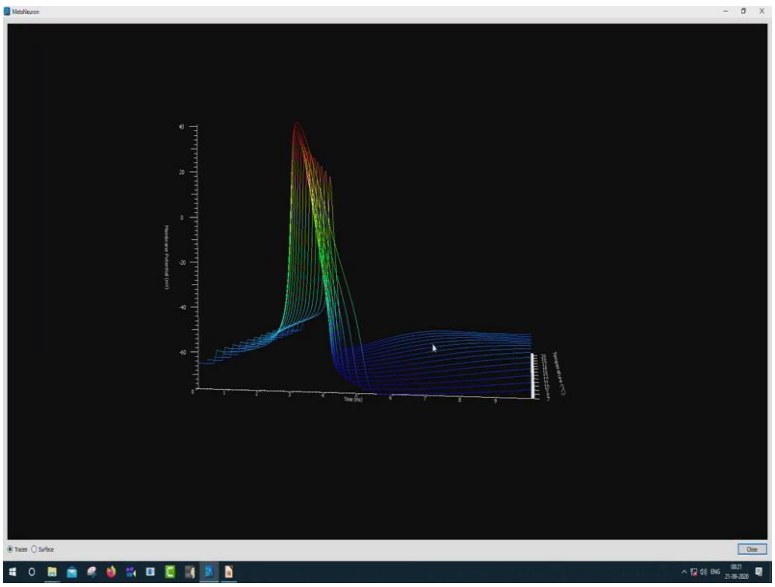
So, when you change the temperature and the range is the begin value is 7, the end value is 21, these are physiological, in the physiological range and you are incrementing it by 1. So, let us change this to something a little more sensible so that you can see this. So, at different temperatures you see what happens and as the temperature changes, the initiation of the action potential also changes.

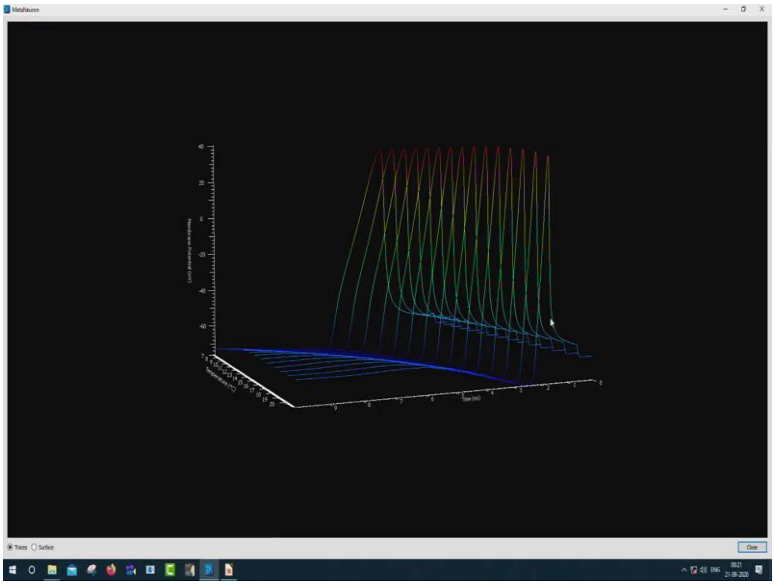
Easier to see with the 3D graph. So, if you look at low temperature, 7, 8, it takes a little time to active and then it comes down and it is much more broad, while if you look at a higher temperature, 20, 21, takes off much faster and comes down baseline much faster.

(Refer Slide Time: 13:17)







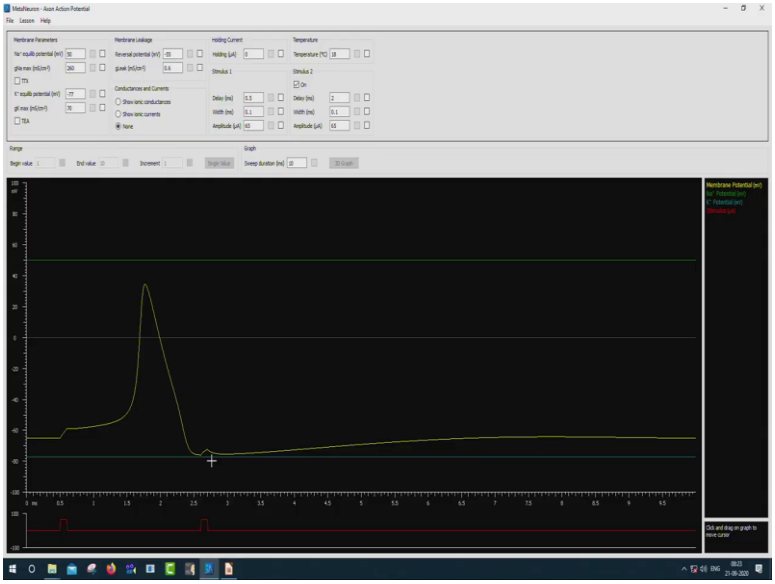


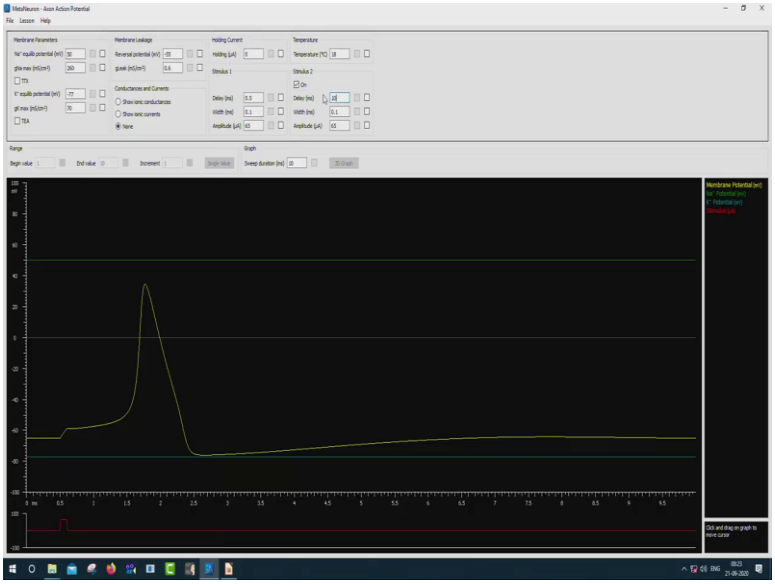
A surface plot is kind of helpful over here. So, you can actually see it. So, this is at a low temperature, and you can see at a higher temperature, it is much narrower, you can see that part over there also, it is much narrower, much narrower over here, much broad over here at low temperature.

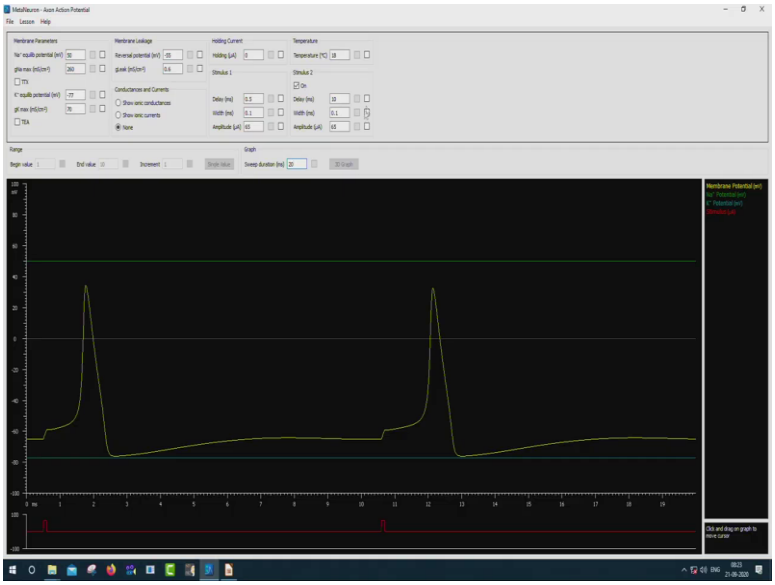
So, a practical point is that when we do experiments, it is critical that we keep the temperature constant and typically with squid, they keep it at 16 degrees and if you are doing mammalian experiments, 18 degrees because when you change the temperature, if it is not held constant, your results will vary considerable as you can see.

And especially if you are recording from a local field potential stimulation a kind of data, it is not just 1 neuron, it is multiple neurons, a whole network and everything changes. So, this is critical and the other thing is as I mentioned earlier one of the ways of treating multiple Sclerosis is lower the temperature, so the thresholds go up. All this is given in great detail in the papers as well as the exercises. So, I encourage you to check it out. And I will just restore things to baseline.

(Refer Slide Time: 14:46)



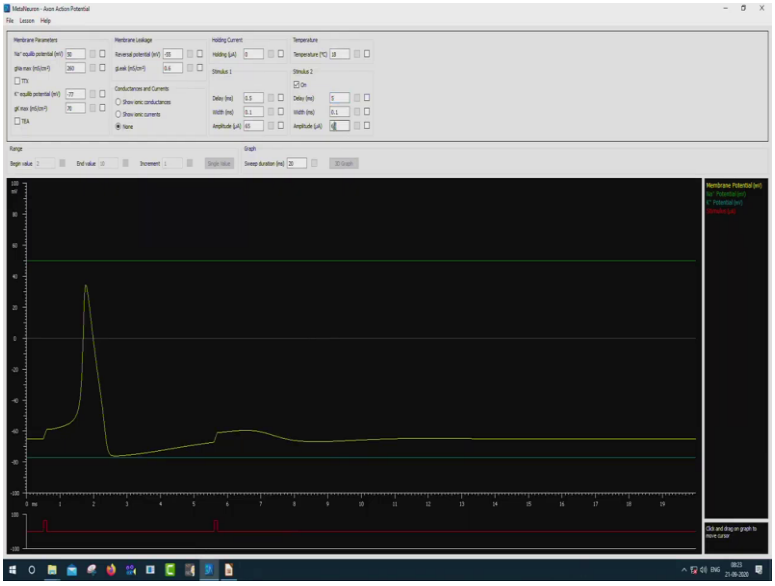


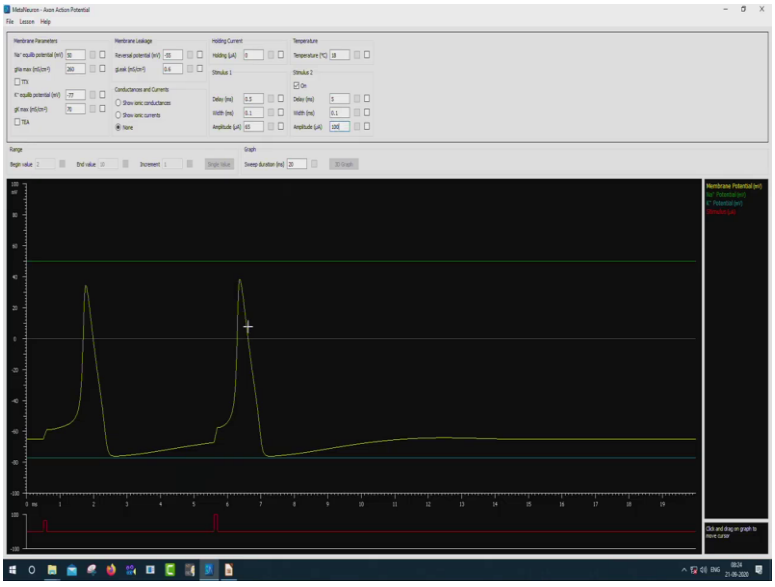


And there is one final thing that I would like to show you in the action potential demo. We will change this again to 10 and then I will put on another stimulus. So, you have one stimulus giving rise to one action potential so I put another stimulus over here and now, you have nothing and you have nothing because this is during the absolute refractory period of the axon. So, it will not fire, the sodium inactivation prevents it from doing that.

But what happens if I change the delay? So, I change the delay to 10, we cannot see it because it is over there, let us make this 20, so I have changed the delay to 10, so 10 meaning 10 from here, from here to here it is 10 and then you see the action potential being fired by the second stimulus. And what happens if I get it to an intermediate path?

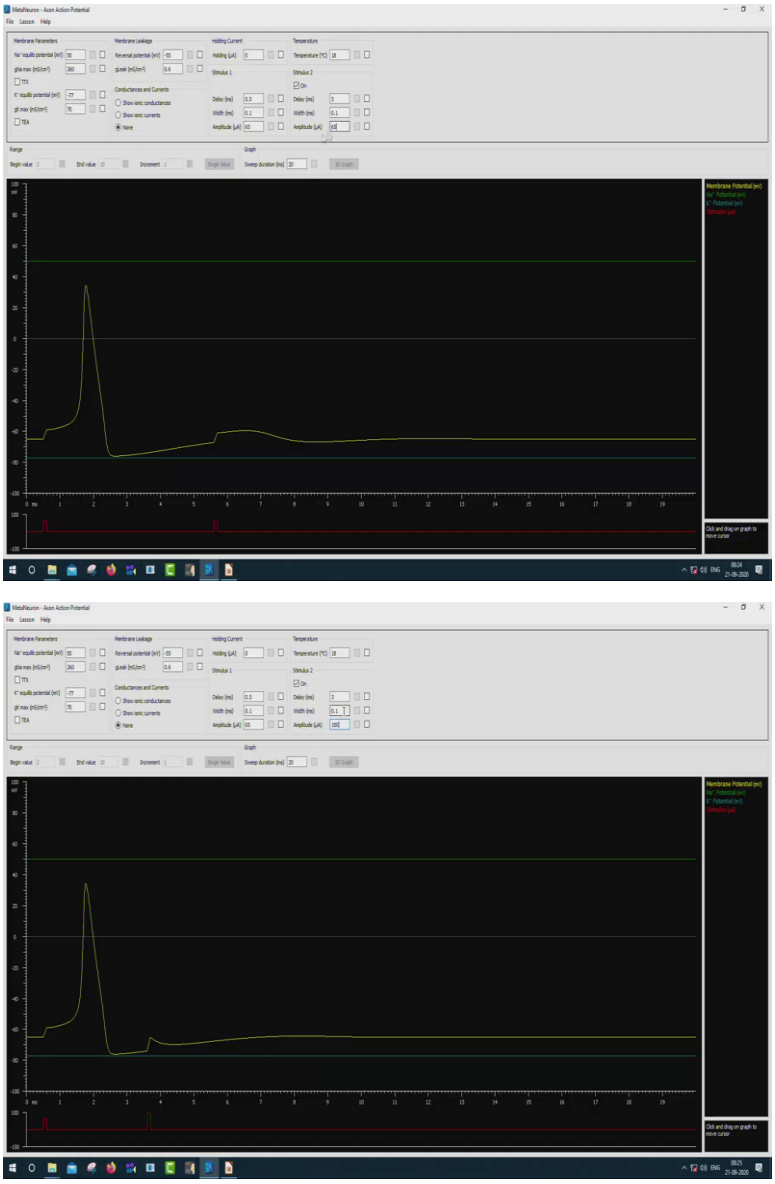
(Refer Slide Time: 15:46)





So, this is where the range kind of helps. So, we will, no we will not do that, what we will do is we will change the delay to 7, you still see it, to 5, there you do not. But in 5 if you increase the amplitude to 100, you get it. So, what we have done here is, we have forced the axon to fire again even though it is refractory, but this is the relative refractory period. So, with increase in current, amplitude of the current, it fires.

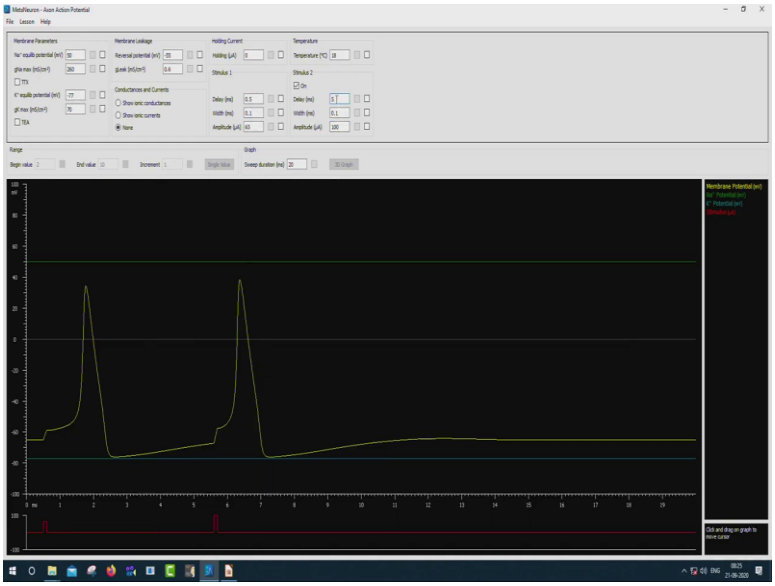
(Refer Slide Time: 16:22)

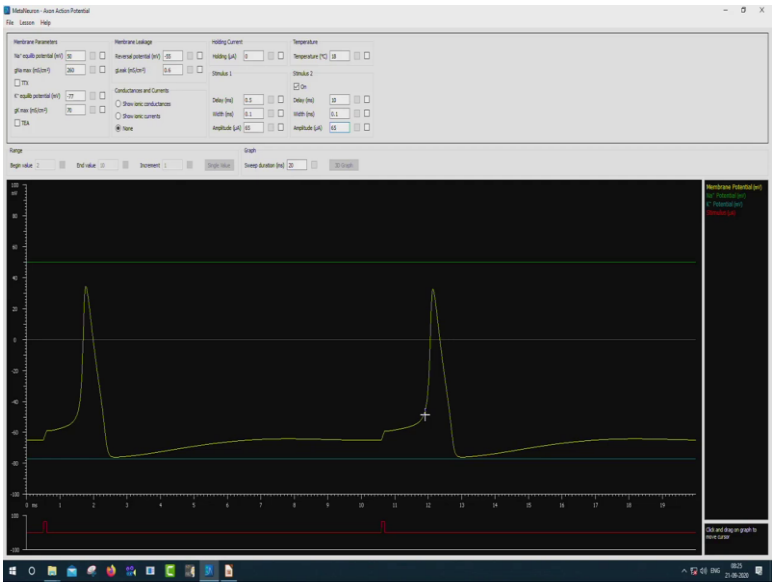


But if it goes, if you use the standard current like here, it does not. But there is a limit, when you make it even shorter, the delay to maybe 4 or maybe 3, so now even if you increase this to 100, it does not fire. So, this is a very important concept that once an axon fires, it takes some charge, sometime to recharge the capacitance because the charge of the resting membrane potential lies on either side of the cell membrane, so it takes some time and there

is a period where it is absolutely refractory regardless of how high you may get the stimulus, it will not fire.

(Refer Slide Time: 17:22)





And after some time it will fire but you need a higher threshold, the amplitude needs to be higher and after about 10, it comes back to normal. So, with normal amplitude, it fires. Notice that when I change the amplitude from 100 to 65, it was slightly delayed so, the thresholds have changed.

So, I encourage you to play with, you have so many parameters to play with, so if you get nothing else from the course, if you get an idea of how the action potential works, that would be really great.