Introductory Neuroscience & Neuro-Instrumentation
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Lecture - 5
Electrophysiological Recordings

So introductory Neuroscience & Neuro-instrumentation, Electrophysiological Recordings.

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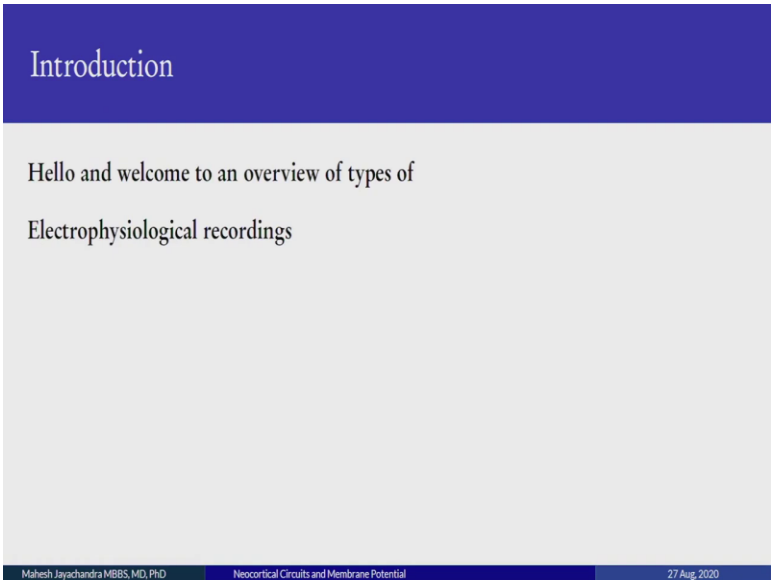

Hello and welcome to an overview of types of electrophysiological recordings.

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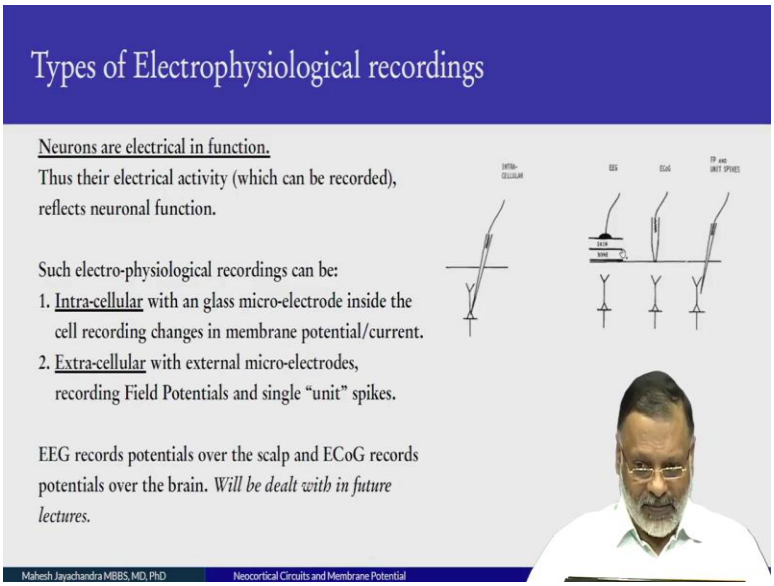

Neurons are electrical in function, that is what we have gathered so far. And thus, their electrical activity if we can record it, it reflects neuronal function. Now there are different types of electrophysiological recordings.

You have intracellular recordings, where you have a glass microelectrode inside the cell recording changes in membrane potential or current, like so. And then, you have extracellular recordings, where the electrode is outside the cell and there you get the extracellular activity of the cell and it is called units or spikes.

Now, this can be really close to the cell, it can be on the surface of the brain, electrocorticogram, or it can be EEG, electroencephalogram, where you record from the scalp, the skin, the bone and then through all that you sense the electrical activity. So electrocorticograms and EEG we will discuss in future sessions.

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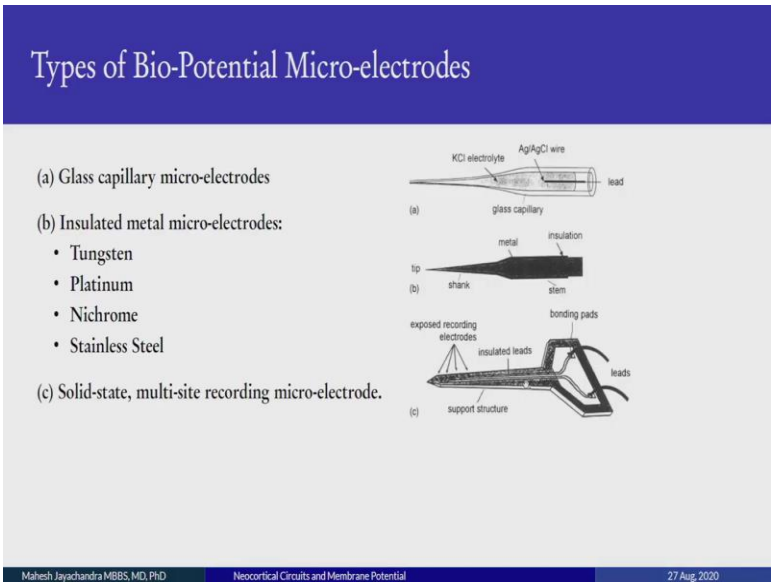

let us talk about the actual techniques, the microelectrodes which you need to record. The most used variety of electrodes are glass capillary microelectrodes. And here, you have a glass capillary tube, and one part is heated and pulled so that the tip becomes in the micron, 10-micron range.

Now that is filled with a high molar potassium chloride solution to give electrical conductivity to a silver-silver chloride wire which goes to the electronics. At the tip, you have electro diameters at the micron level, it could be 1 micron, 5, 10 so on, and so forth.

Alternatively, you could use metal microelectrodes. So typically, the metals used are tungsten, platinum, nichrome, and stainless steel because they are all very stiff. So here you electro point, you pass AC current, for example, through the electrode and it connects with the circuit and with that, you can make very fine tips at the micron level.

And you have the metal, it is insulated with something suitable like foam wire and only the tip is exposed. The glass and the metal microelectrodes are the workhorses of neurophysiology, they have been with us for the last 50, 100 years.

Lately, you have something called solid-state electrodes can be fabricated from silicon substrates and this allows us to fabricate electrodes depending on the structure of the

neurons, the structure of the neuronal area we are recording from. In fact, this is being done at the Institute of Science in Professor Hardik Pandya's lab.

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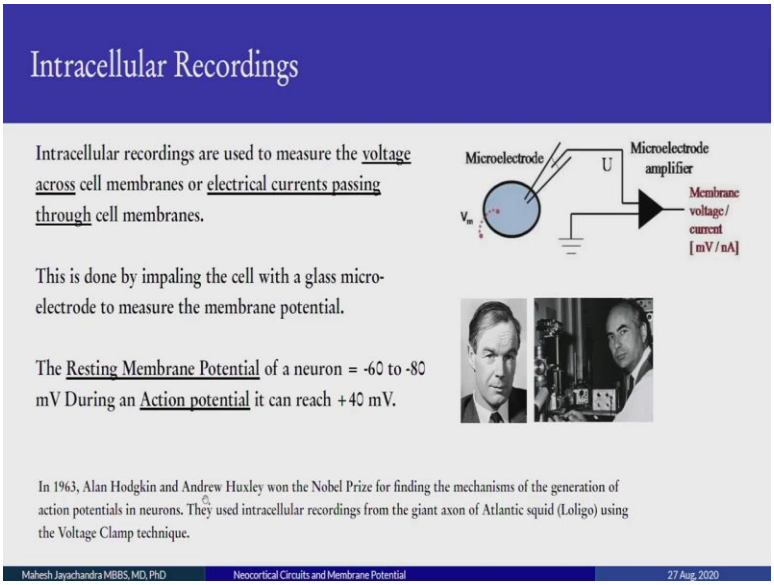

let us consider intracellular recordings. So typically, you have a cell, you have a microelectrode which we showed, a glass microelectrode that impales the cell, and then between inside and outside you record the potential. The microelectrode is connected through the silver-silver chloride wires to a microelectrode amplifier and then it goes to the signal conditioning circuits which either record the voltage of the currents.

When you do this, as soon as you insert the electrode inside the cell, you get a potential. Now, this potential is typically minus 60 to minus 80 millivolts with respect to the outside, so when there is activity of the nerve, we call it the action potential, which we will study in-depth in a future session it can reach up to 40 millivolts, plus 40 millivolts.

Alan Hodgkin and Andrew Huxley did a series of seminal experiments in the 40s and the 50s where they developed new techniques to find out the mechanism of generation of the action potential. For this, they won the Nobel Prize in 1963. These intracellular electrodes from were used from the giant axon of the squid.

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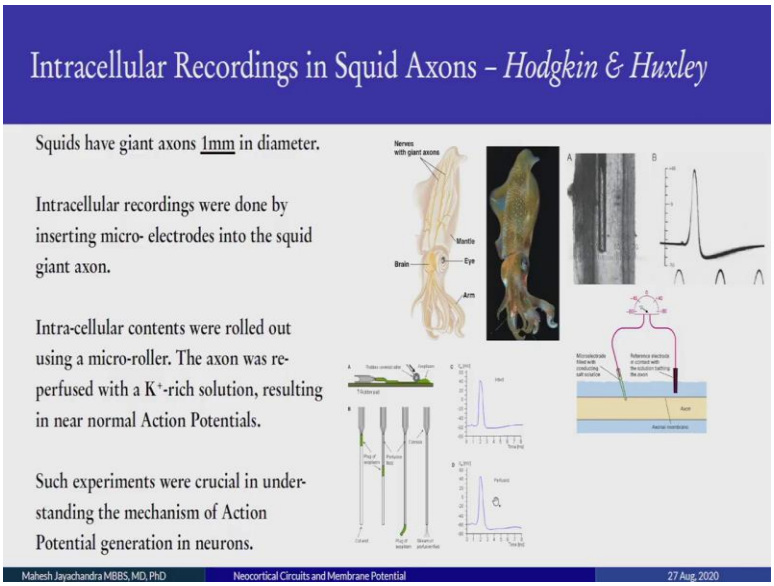

So this is a squid. It is a deep-sea dwelling creature, and we are not really interested in it except in these two giant axons. Now, these are very big and the largest in the animal kingdom nothing bigger has been shown.

They are 1 millimeter in diameter and this allows us, this dimensions of this axon allows us to put in, insert a glass microelectrode inside as you see over here and the circuit is what we showed, this is a simplified version of the circuit shown in the previous slide where you have the electrode inside the axon and then you have the signal conditioning circuit and the ground electrode will be outside in the extracellular fluid.

So, this is the original recording, this part over here is where the electrode has been inserted into the axon and then a little bit of current is injected into the circuit and then it has an action potential. This is the resting membrane potential and this is action potential and this highly dependent on intracellular potassium.

The way they proved it was they did a very elegant experiment, they rolled out the cytoplasm using a road roller and they re-perfused the axon with a high potassium solution and lo and behold, this is the action potential, the resting membrane potential, the action potential with the intact axon; and this is the resting membrane position, action potential with the re-perfused axon, re-perfused with high potassium.

So this brings out a very important point that the action potential and the membrane potential of the axon, it is due to the potassium and the ionic concentration and the other subcellular components of the cell if you will, they are not really involved in generation of the resting membrane and the action potential. These experiments were crucial in understanding the mechanism of action potential generation in neurons.

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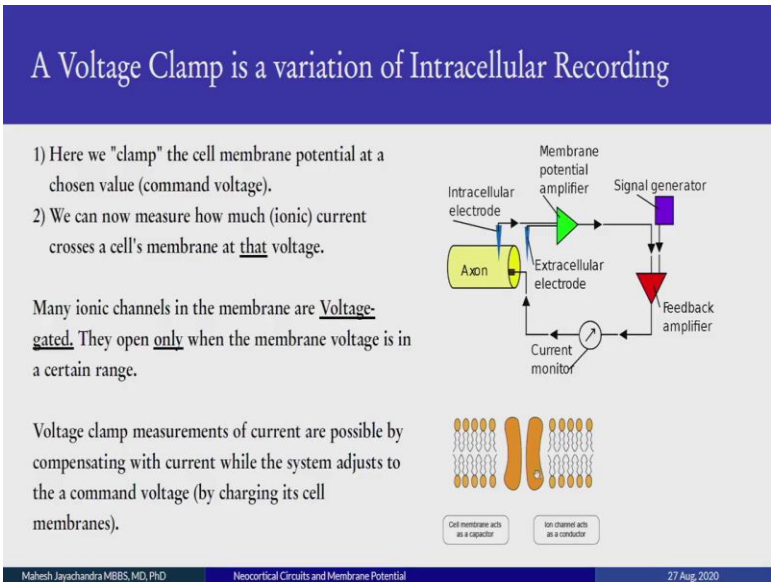

Further, they developed, Hodgkin and Huxley developed a voltage clamp technique which is a variation of the intracellular recording. Here we have the electrode inside, and we clamp the cell at a particular voltage, a command voltage it is called. And at that voltage, with the appropriate signal conditioning circuit, we record how much current crosses the membrane.

Now, remember this is ionic current. In biology, neurophysiology charges are transferred by ions and not by electrons. Many ionic channels in the membrane are voltage-gated, which means they open only at a particular range, within a certain range and this is a schematic of the cell membrane.

You have the phospholipid layer, a cell membrane which we studied earlier and that acts as a capacitor and then started in the phospholipids sea as it were, you have these icebergs, ionic channels floating and that at a particular voltage range opens, and that acts

as a conductor. When we clamp the axon at a particular voltage, enough current has to be injected to balance this command voltage by charging its cell membranes.

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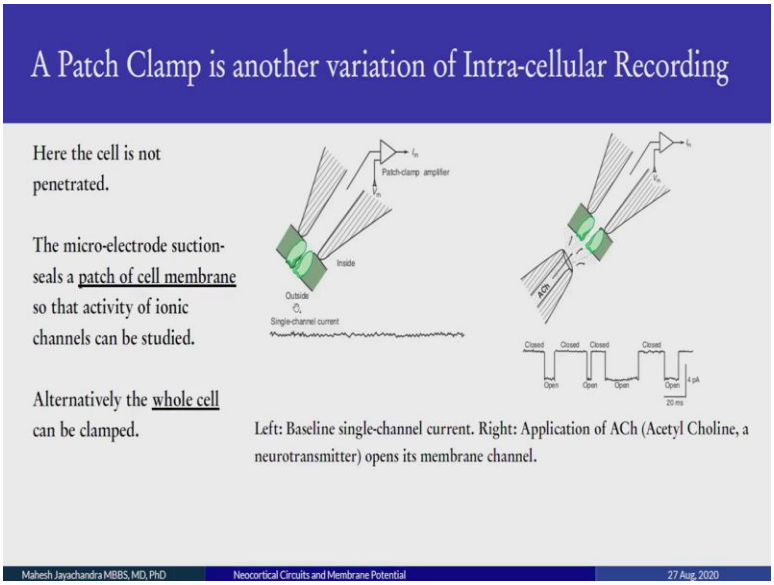

A patch-clamp is another variation of intracellular recordings. Here the cell is not penetrated the microelectrode, using a suction apparatus, is patched to a bit of cell membrane which has the channel of interest, and then you have the usual electronics.

And here, you see the current at rest when the channel is closed you have this baseline. And then when we speak of spritz, an appropriate neurotransmitter on the channel, the channel opens and it either opens or closes quantal in that way. You can see it closed and then it opens, then it is closed again, then it is open, and so on and so forth.

Alternatively, the whole-cell membrane potential and currents can be recorded by clamping the whole-cell without focusing on a single patch or a single ion, this gives recordings equivalent to intracellular recordings of the cell with the advantage that the cell is not damaged because when you impale something there is damage and then the life of the cell, recording life it gets low. When you patch the whole cell, you can record for longer periods of time.

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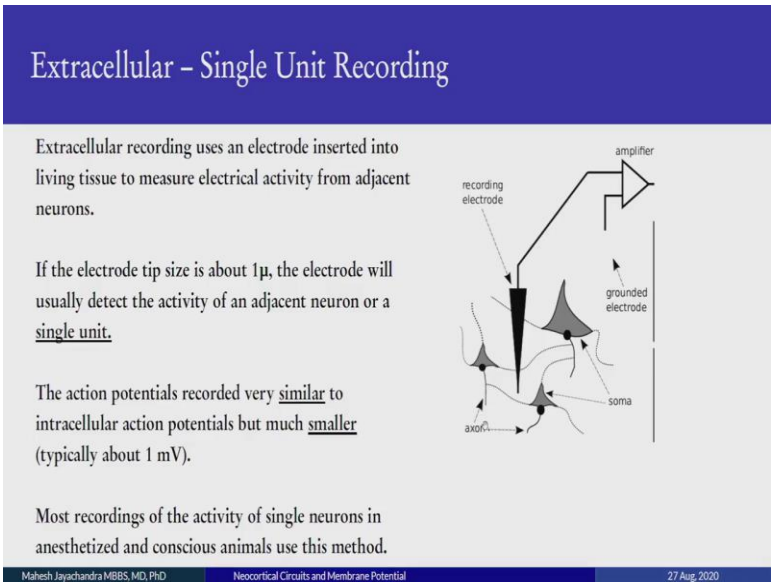

Let us consider extracellular recordings. As we had mentioned, here the electrode is very close to the neuronal tissue, it is not inside the neuronal tissue and it is just adjacent to it. If it is about 1µm, 1 micron, the electrode tip, it would record the activity of an adjacent cell and that is called a spike or a single unit.

Now, these are very similar to intracellular action potentials but much smaller, typically, only about 1 millivolt but they can be picked up. And many, many experiments, thousands of experiments in fact, in animals, in conscious and anesthetized animals, have used this technique, simply because this is a much more robust technique in the sense that the animal can move and you can still record the spikes.

If it is inside the cell and even slight movement then either the nerve gets damaged or the electrode slips out of the cell. There are certain advantages of extracellular recording.

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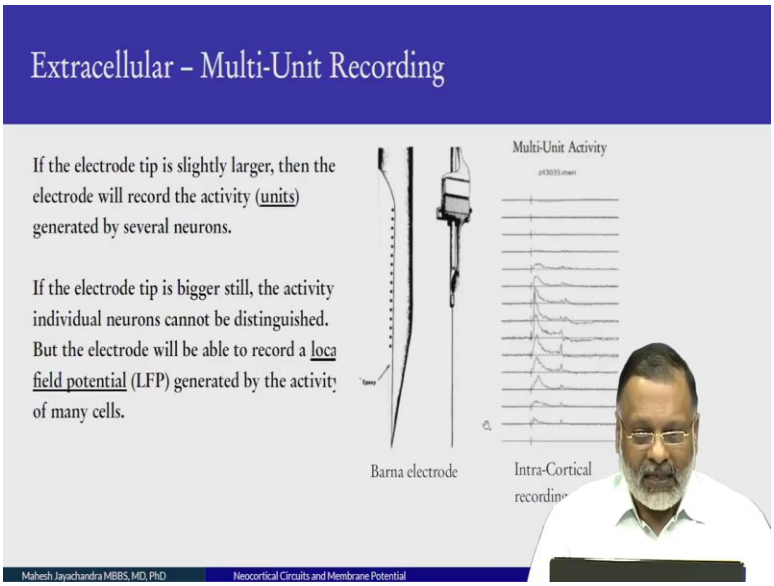

If it is slightly larger, maybe about 5 or 10 microns, then you start getting multiple unit activity, activity of units which are you do not, you cannot distinguish individual units but you see a population of units.

So these are recordings from the auditory cortex of a monkey and this was the electrode which was used, which is a linear array of 15, 16 microelectrodes separated by 100 mu and it is called the Barna electrode and this records multi-unit activity. And if it is bigger still and if you change the filter settings then you record local field potentials.

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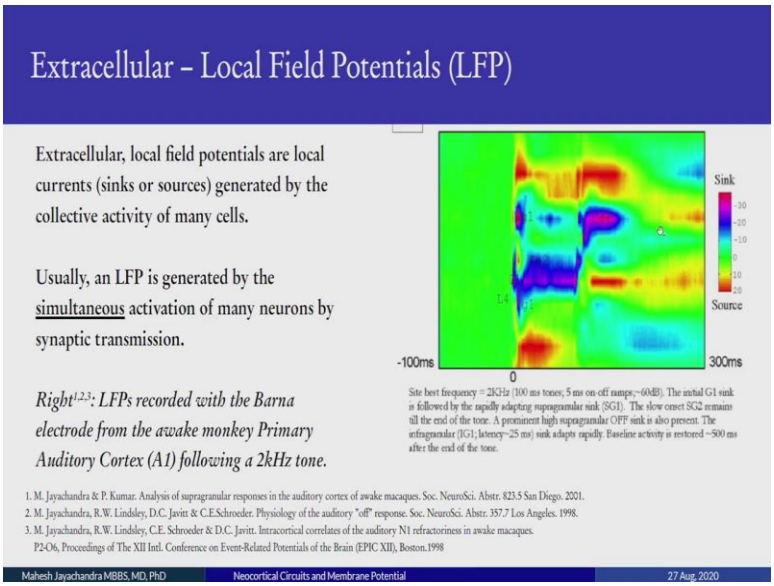

This is the field potentials recorded when an ensemble or a neuronal circuit gets activated. So here you see the Barna electrode being used to record activity in the auditory cortex of the monkey, in the auditory area, the response to a tone. And you can actually see the tone over here, the tone is a 100-millisecond tone and these are the different areas layers of the cortex which we should lead with subsequently, but this is a local field potential.

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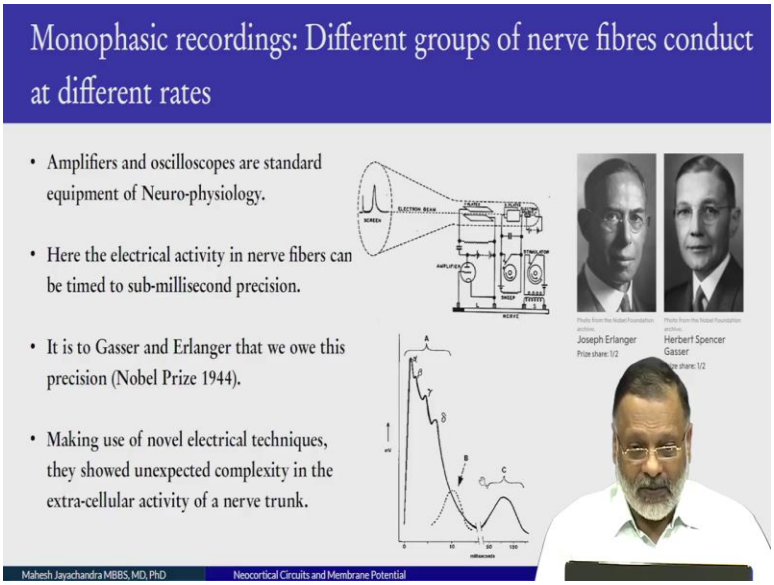

The final kinds of recordings that we will consider are monophasic recordings. Here, these are a variation of extracellular recordings by different groups of nerve fibers conduct a different rate and we can see that using this technique.

A little bit of history. Amplifiers and oscilloscopes are standard equipment in neurophysiology. In the old days, you had equipment like this and they were all based on valves, radio valves, and tubes and cathode-ray oscilloscopes. Now, of course, you have solid-state mechanism, methods and you also have computers instead of oscilloscopes, but the fundamental principles remain the same.

The electrical activity in the nerves can be timed to sub-millisecond precision and it was to Joseph Erlanger and Herbert Gasser we owe these increase in instrumentation advances and they applied it immediately to looking at the electrical activity in peripheral nerves, so and they found that a peripheral nerve has different populations of neurons, a sensory nerve.

It has populations for pain, it has populations of nerves for temperature, pressure, cold, so on, and so forth and when they use this technique they found that they could discern these populations easily, and for this work, they won the Nobel Prize in 1944.

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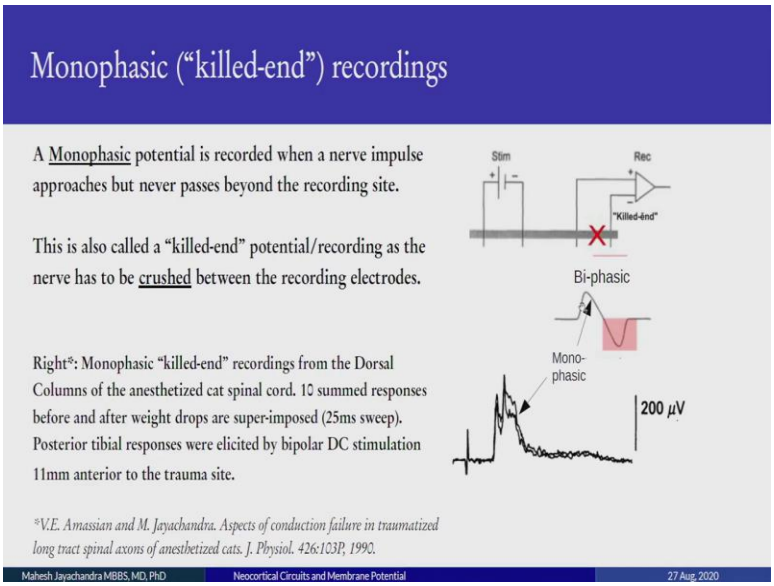

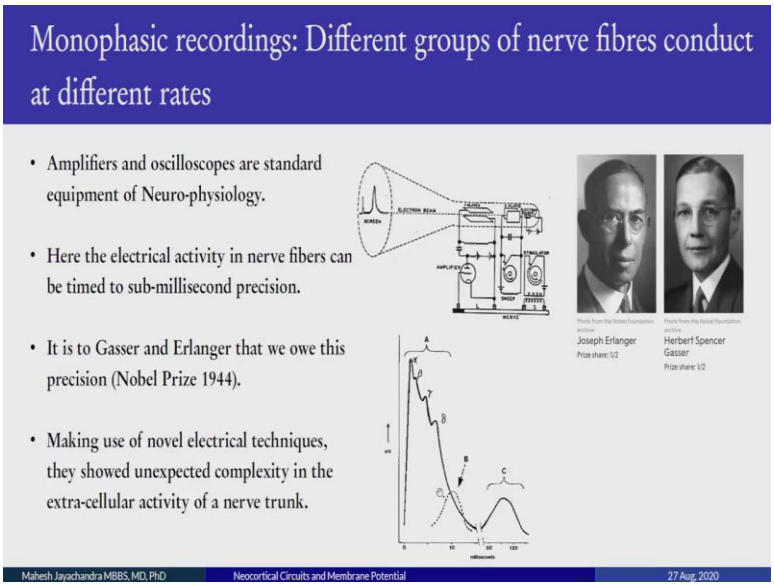

What is the Monophasic killed-end recording? This is a potential which is recorded when a nerve impulse approaches but never passes beyond the recording site. So here, you have a stimulus, this is the nerve, and this is the recording site.

And typically, in a biphasic recording way, up and down, the electrical activity comes here and then goes here in the opposite direction, so you have a biphasic response. But if you crush the nerve in between, and that is why you get the name killed-end recording,

you only get half of it, which is a monophasic response. And this is a monophasic response which Gasser and Erlanger used.

So on the right over here, are monophasic responses from a cat Dorsal Column which is on the spinal cord and this is from my thesis where I was looking at the activity of the sensory columns to trauma. And this is still an important technique which can be used experimentally.

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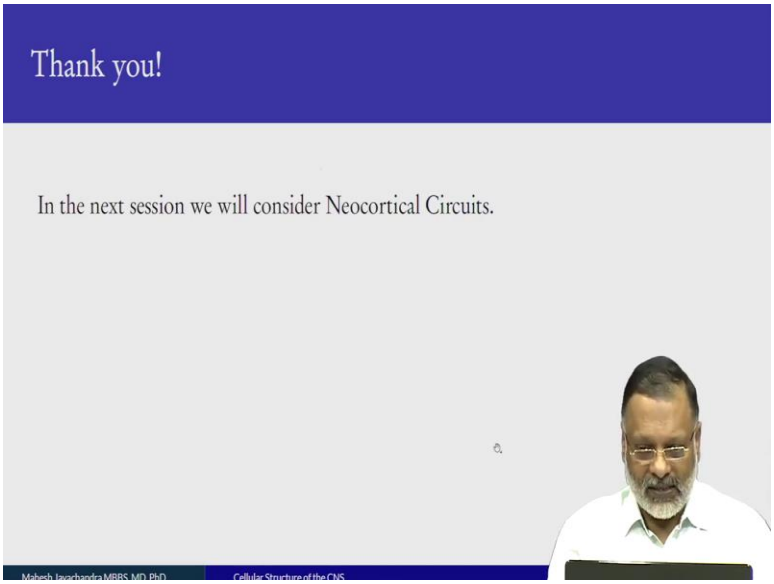

So thank you. In the next session, we shall consider Neocortical Circuits.