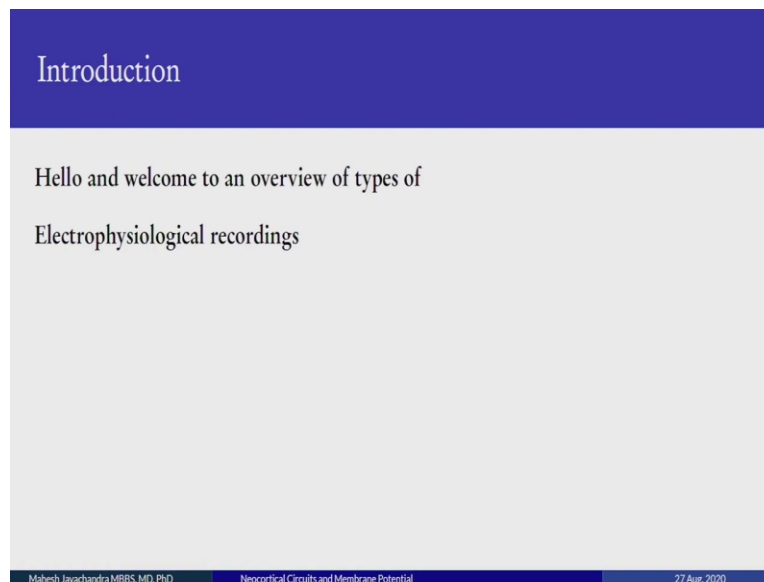


Introductory Neuroscience & Neuro-Instrumentation
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Lecture - 5
Electrophysiological Recordings

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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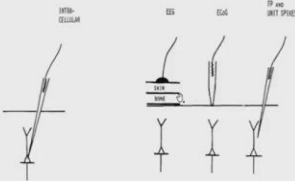
Types of Electrophysiological recordings

Neurons are electrical in function.
Thus their electrical activity (which can be recorded), reflects neuronal function.

Such electro-physiological recordings can be:

1. Intra-cellular with an glass micro-electrode inside the cell recording changes in membrane potential/current.
2. Extra-cellular with external micro-electrodes, recording Field Potentials and single "unit" spikes.

EEG records potentials over the scalp and ECoG records potentials over the brain. *Will be dealt with in future lectures.*



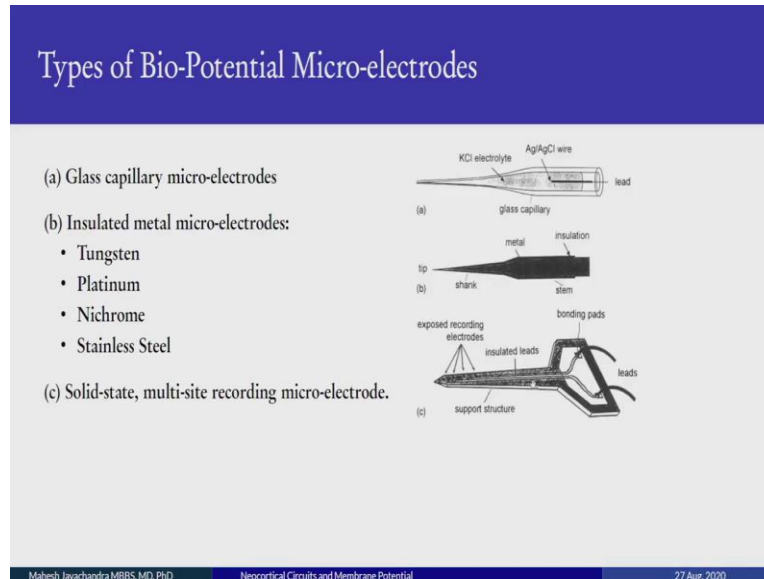
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Neurons are electrical in function, that is what we have gathered so far. And thus, their electrical activity if we can record it, it reflects neuronal function. Now there are different types of electrophysiological recordings.

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 electrode 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.

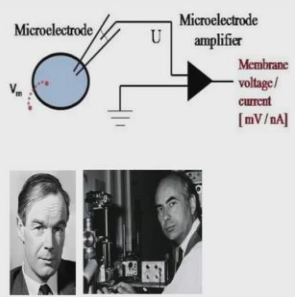
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Intracellular Recordings

Intracellular recordings are used to measure the voltage across cell membranes or electrical currents passing through cell membranes.

This is done by impaling the cell with a glass micro-electrode to measure the membrane potential.

The Resting Membrane Potential of a neuron = -60 to -80 mV. During an Action potential it can reach $+40$ mV.



In 1963, Alan Hodgkin and Andrew Huxley won the Nobel Prize for finding the mechanisms of the generation of action potentials in neurons. They used intracellular recordings from the giant axon of Atlantic squid (Loligo) using the Voltage Clamp technique.

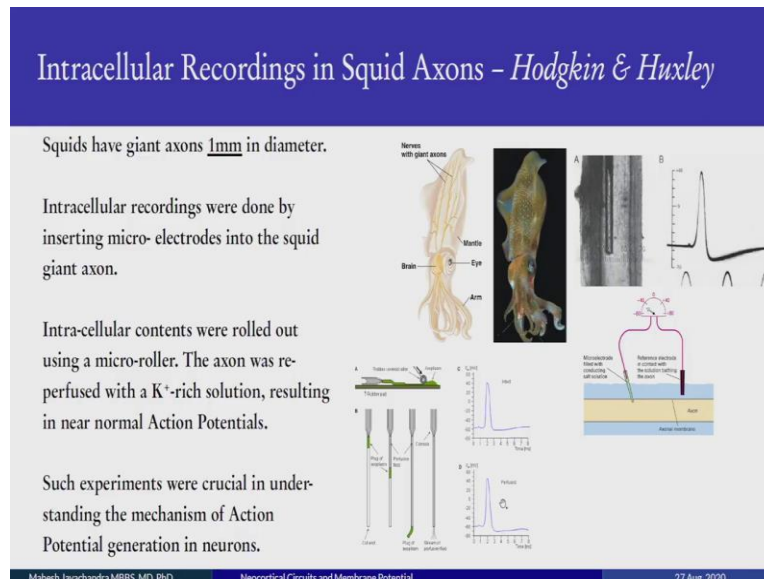
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let us consider intracellular recordings. So typically, you have a cell, you have a microelectrode which we showed, a glass microelectrode that impales the cell, and then between inside and outside you record the potential. The microelectrode is connected 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.

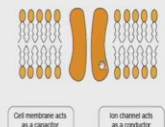
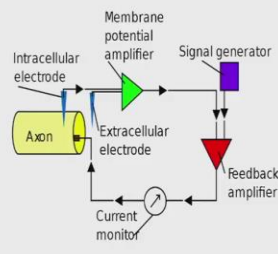
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A Voltage Clamp is a variation of Intracellular Recording

- 1) Here we "clamp" the cell membrane potential at a chosen value (command voltage).
- 2) We can now measure how much (ionic) current crosses a cell's membrane at that voltage.

Many ionic channels in the membrane are Voltage-gated. They open only when the membrane voltage is in a certain range.

Voltage clamp measurements of current are possible by compensating with current while the system adjusts to the a command voltage (by charging its cell membranes).



Cell membrane acts as a capacitor

Ion channel acts as a conductor

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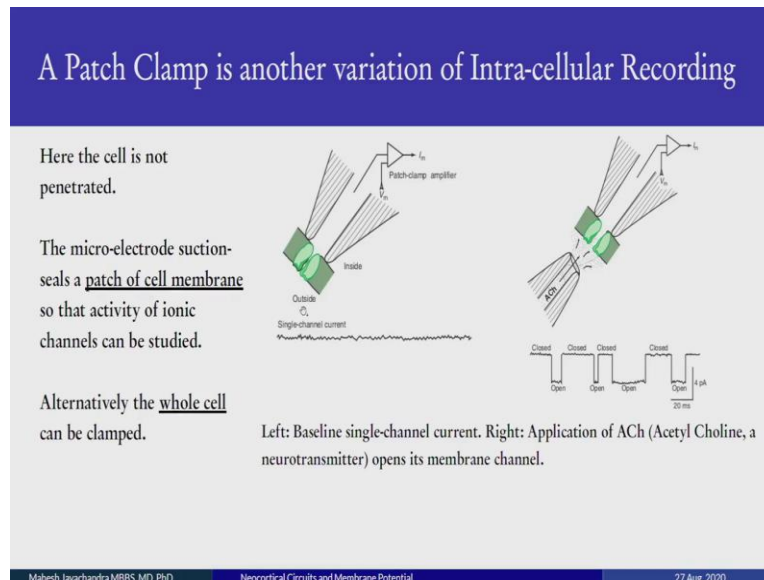
Further, they developed, Hodgkin and Huxley developed a voltage clamp technique which is a variation of the intracellular recording. Here we have the electrode inside, and we clamp the cell at a particular voltage, a command voltage it is called. And at that voltage, with the appropriate signal conditioning 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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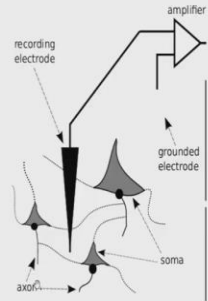
Extracellular – Single Unit Recording

Extracellular recording uses an electrode inserted into living tissue to measure electrical activity from adjacent neurons.

If the electrode tip size is about 1μ , the electrode will usually detect the activity of an adjacent neuron or a single unit.

The action potentials recorded very similar to intracellular action potentials but much smaller (typically about 1 mV).

Most recordings of the activity of single neurons in anesthetized and conscious animals use this method.



The diagram illustrates the setup for extracellular single unit recording. A recording electrode, shown as a sharp needle-like tip, is inserted into the tissue near a neuron. The neuron is depicted with its soma and axon. A grounded electrode is also shown, connected to a common ground. The recording electrode is connected to an amplifier, which is represented by a triangle symbol. The axon is labeled 'axon' and the soma is labeled 'soma'.

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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 $1\mu\text{m}$, 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.

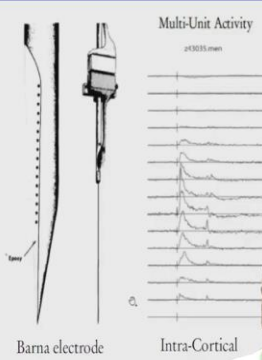
If it is inside the cell and even slight movement then either the nerve gets damaged or the electrode slips out of the cell. There are certain advantages of extracellular recording.

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Extracellular - Multi-Unit Recording

If the electrode tip is slightly larger, then the electrode will record the activity (units) generated by several neurons.

If the electrode tip is bigger still, the activity individual neurons cannot be distinguished. But the electrode will be able to record a local field potential (LFP) generated by the activity of many cells.



The diagram shows a Barna electrode, which is a linear array of microelectrodes. To its right, there are two sets of recordings. The top set, labeled 'Multi-Unit Activity', shows several distinct, sharp action potential spikes. The bottom set, labeled 'Intra-Cortical recording', shows a single, broad, low-amplitude waveform representing a local field potential (LFP). A scale bar for the LFP recording indicates 1000 mV and 100 ms.

Barna electrode

Multi-Unit Activity

1000 mV

100 ms

Intra-Cortical 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 μ m 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.

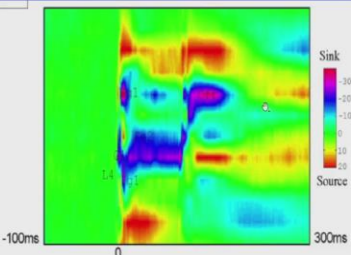
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Extracellular – Local Field Potentials (LFP)

Extracellular, local field potentials are local currents (sinks or sources) generated by the collective activity of many cells.

Usually, an LFP is generated by the simultaneous activation of many neurons by synaptic transmission.

Right^{1,2,3}: LFPs recorded with the Barna electrode from the awake monkey Primary Auditory Cortex (A1) following a 2kHz tone.



Site best frequency = 2KHz (100 ms tone, 5 ms on off ramps, -60dB). The initial G1 sink is followed by the rapidly adapting supragranular sink (SG1). The slow onset SG2 remains all the end of the tone. A prominent high supragranular OFF sink is also present. The infragranular (IG), latency ~25 ms) sink adapts rapidly. Baseline activity is restored ~500 ms after the end of the tone.

1. M. Jayachandran & P. Kumar. Analysis of supragranular responses in the auditory cortex of awake macaques. Soc. Neurosci. Abstr. 823.5 San Diego, 2001.
2. M. Jayachandran, R.W. Lindsay, D.C. Jaritt & C.E. Schroeder. Physiology of the auditory "off" response. Soc. Neurosci. Abstr. 357.7 Los Angeles, 1998.
3. M. Jayachandran, R.W. Lindsay, C.E. Schroeder & D.C. Jaritt. Intracortical correlates of the auditory N1 refractoriness in awake macaques. P2-06, Proceedings of The XII Intl. Conference on Event-Related Potentials of the Brain (EPIC XII), Boston, 1998


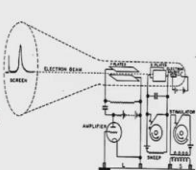
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This is the field potentials recorded when an ensemble or a neuronal circuit gets activated. So here you see the Barna electrode being used to record activity in the auditory cortex of the monkey, in the auditory area, the response to a tone. And you 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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Monophasic recordings: Different groups of nerve fibres conduct at different rates

- Amplifiers and oscilloscopes are standard equipment of Neuro-physiology.
- Here the electrical activity in nerve fibers can be timed to sub-millisecond precision.
- It is to Gasser and Erlanger that we owe this precision (Nobel Prize 1944).
- Making use of novel electrical techniques, they showed unexpected complexity in the extra-cellular activity of a nerve trunk.



Joseph Erlanger
Nobel Prize 1944

Herbert Spencer Gasser
Nobel Prize 1944

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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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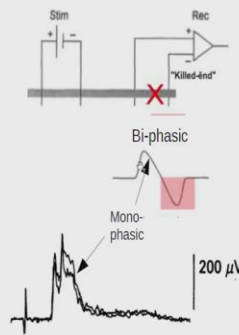
Monophasic (“killed-end”) recordings

A Monophasic potential is recorded when a nerve impulse approaches but never passes beyond the recording site.

This is also called a “killed-end” potential/recording as the nerve has to be crushed between the recording electrodes.

Right*: Monophasic “killed-end” recordings from the Dorsal Columns of the anesthetized cat spinal cord. 10 summed responses before and after weight drops are super-imposed (25ms sweep). Posterior tibial responses were elicited by bipolar DC stimulation 11mm anterior to the trauma site.

*V.E. Amassian and M. Jayachandra. Aspects of conduction failure in traumatized long tract spinal axons of anesthetized cats. J. Physiol. 426:103P, 1990.



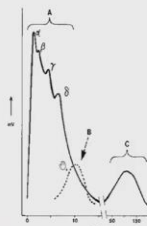
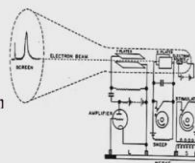
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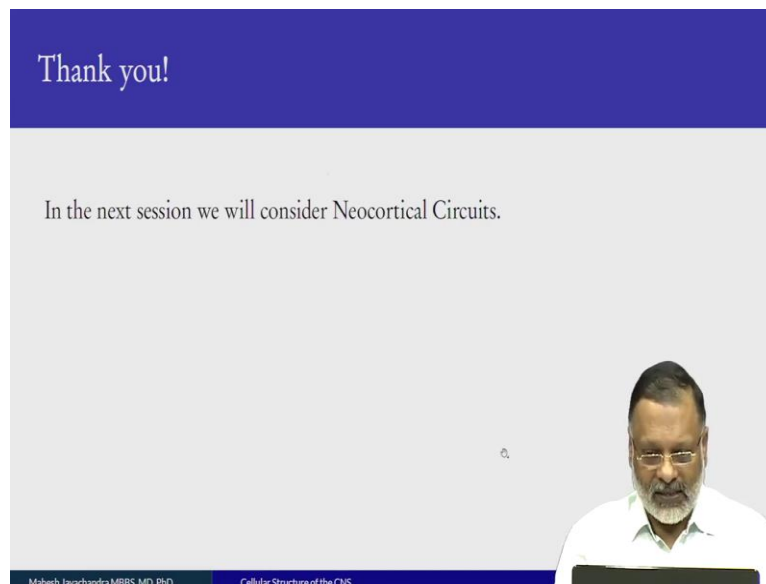
What is the Monophasic killed-end recording? This is a potential which is recorded when a nerve impulse approaches but never passes beyond the recording site. So here, you have a stimulus, this is the nerve, and this is the recording site.

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.