Design for Biosecurity

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Lecture 57

Cell Based Biosensors - Part 2

Welcome back to the class. In our previous session, we explored the neuromuscular junction. Today, I want to give you a more detailed visual on how electrical phenomena are used to study these neuromuscular junctions.

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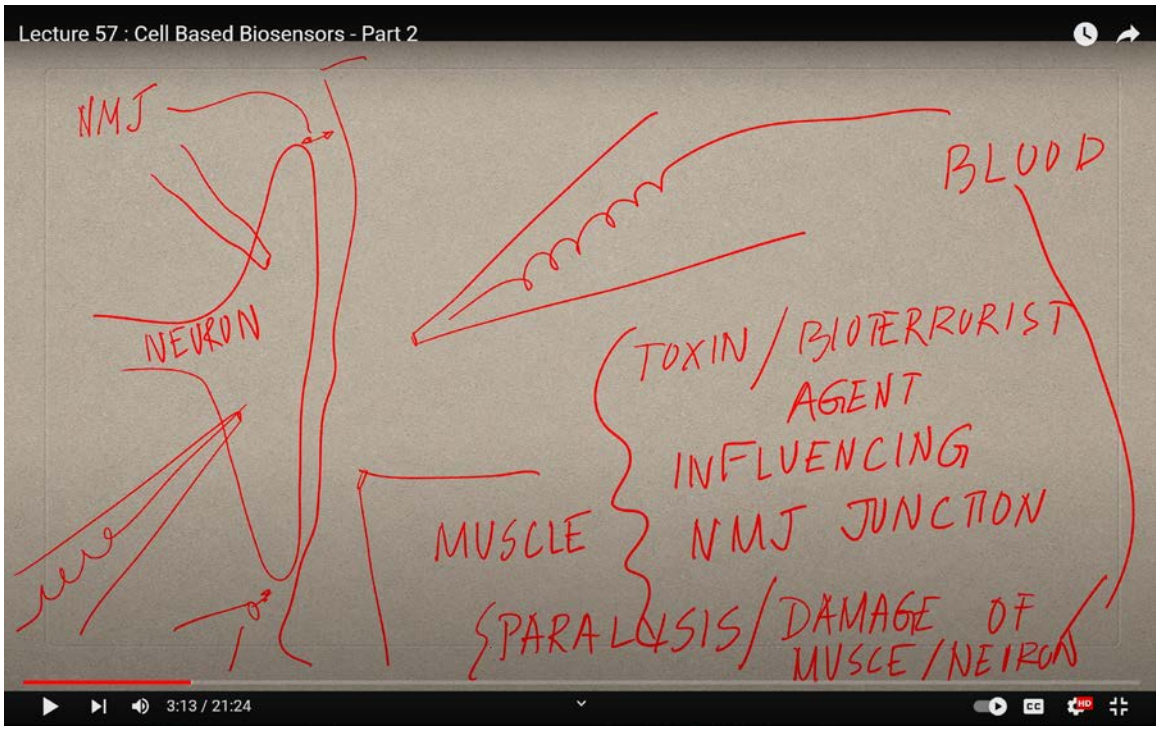

Imagine this is the neuronal end, and over here is the muscle end. The tiny space in between is the neuromuscular junction (NMJ), where communication happens without direct contact. One way to study this is by using patch pipettes. You can place a pipette here and another here, each equipped with electrodes. This technique can range from a triple patch clamp to a quadruple patch clamp, depending on how many sites you're probing. For

example, if this is the muscle site and this is the neuron site, you can inject various compounds into the system for testing.

Let's say you inject a compound under study in this vicinity. By doing so, you can monitor different currents and voltages within this configuration. This approach is one of the most sophisticated biosensing methods available, particularly useful for studying toxins and bioterrorism agents that affect the neuromuscular junction. Historically, many compounds were developed to either paralyze muscles or damage neurons, and in extreme cases, even affect blood chemistry.

When discussing the neuromuscular junction, there are various techniques involving intracellular electrode configurations to better understand these interactions. From here, we will move on to the next topic, extracellular electrodes.

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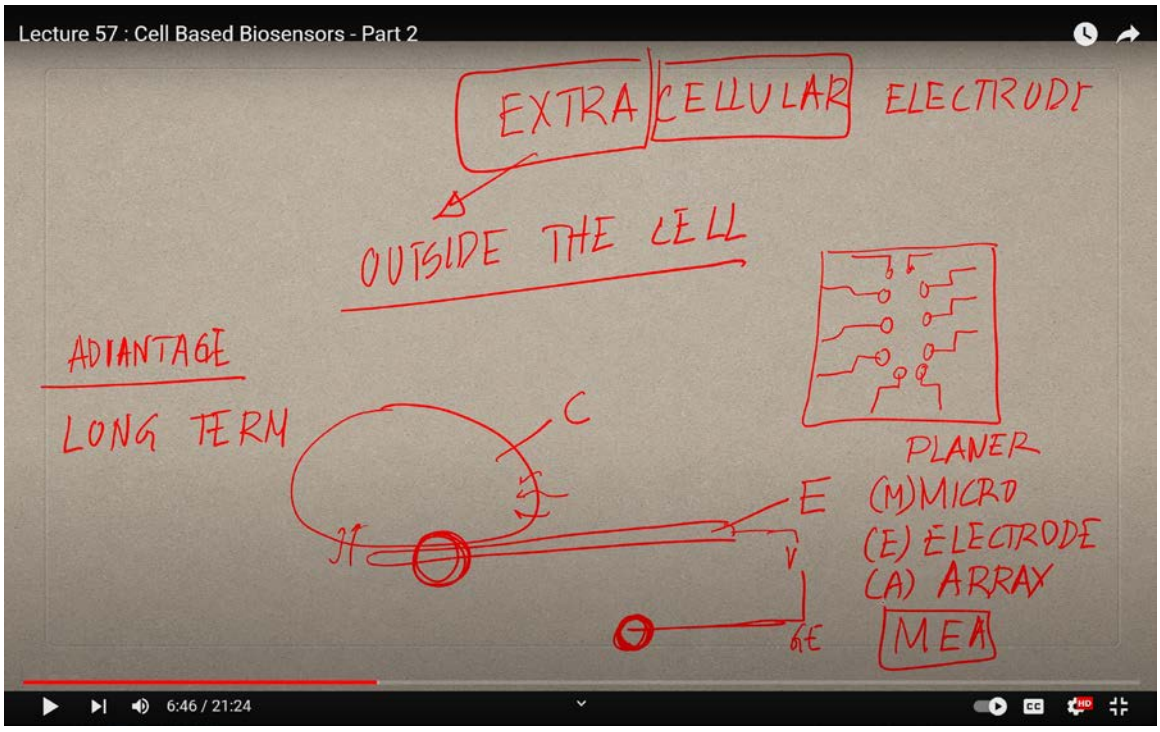

As the name suggests, extracellular electrodes operate outside the cell. In this configuration, you are not invading the cell's interior. If this is the cell, the electrode is placed nearby without disrupting its structure. Imagine this is the cell (C) and this is the

electrode (E). Another electrode, the ground electrode, is positioned nearby. In this setup, you measure the voltage difference between the recording electrode and the ground electrode.

The challenge with extracellular electrodes lies in detecting voltage changes at a distance, but the advantage is that you can conduct long-term studies without damaging the cell. For instance, if you have a planar array of electrodes, where cells are grown on a surface with electrodes embedded beneath them, you can monitor the activity from outside, without interrupting the cell's natural behavior.

This setup is known as a planar microelectrode array (MEA). It's a collection of small electrodes aligned in a grid-like formation, placed under the cells. Though I can't draw its full complexity here, imagine electrodes distributed across a surface with cells sitting on top of them. This allows continuous, non-invasive monitoring.

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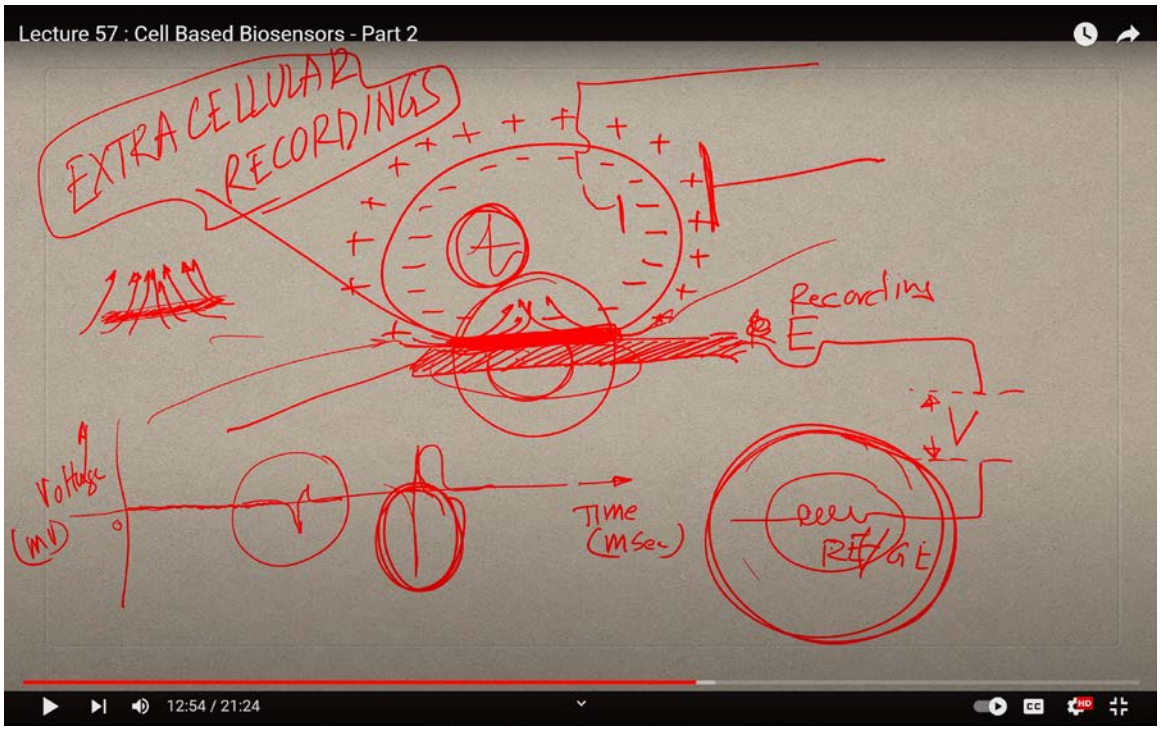

Now, what exactly does this planar microelectrode array do? To understand that, let’s explore what happens at the interface between the cell and the electrode. You have an

electrode (let's mark them with hatch lines), and a ground electrode. Between them, you're measuring voltage. Above this setup, you have an excitable cell. As we know, the inside of the cell is negatively charged, and outside, due to the presence of cations like sodium, it is more positively charged.

If we consider the cell like a battery, an action potential will create a measurable electrical event. At baseline, the voltage appears stable. The y-axis represents voltage (in millivolts) and the x-axis shows time (in milliseconds), and you observe a flat baseline when there is no activity. But when this cell fires an action potential, you will see a spike on this baseline.

At that moment, sodium ions rush into the cell, altering the charge at that specific location. This influx of sodium ions represents the electrical event, which is captured as a sudden voltage change by the electrodes. This is what we're studying when we observe the action potential firing from an excitable cell, such as a neuron or muscle fiber.

So, that's the principle behind using extracellular electrodes and planar microelectrode arrays to study electrical events in cells. This method allows for non-invasive, long-term observations of cells in action, making it a powerful tool for research in biosecurity and neuromuscular physiology.

Alright. So, momentarily, this recording electrode, in comparison to the ground electrode (which we also refer to as the reference or control electrode), will experience a transient negative potential. This happens because the recording electrode is detecting activity, while the reference electrode remains in a region where no electrical events are occurring. As a result, you will observe a dip in the recorded signal, something like this. Once this movement occurs, sodium ions will diffuse and balance the charge, causing the signal to return to its baseline. Initially, you'll see this dip, followed by a return to equilibrium, which may look something like this if we were to magnify it.

This dip signifies that an electrical event is occurring. The electrode momentarily senses a more negative potential relative to the reference electrode because, at this location, there's active diffusion, unlike the reference zone, which remains free from such activity. This is the essence of the signal captured in extracellular recordings. However, it's important to

note that these recordings measure changes in voltage, not the exact current itself. This is where current technology stands.

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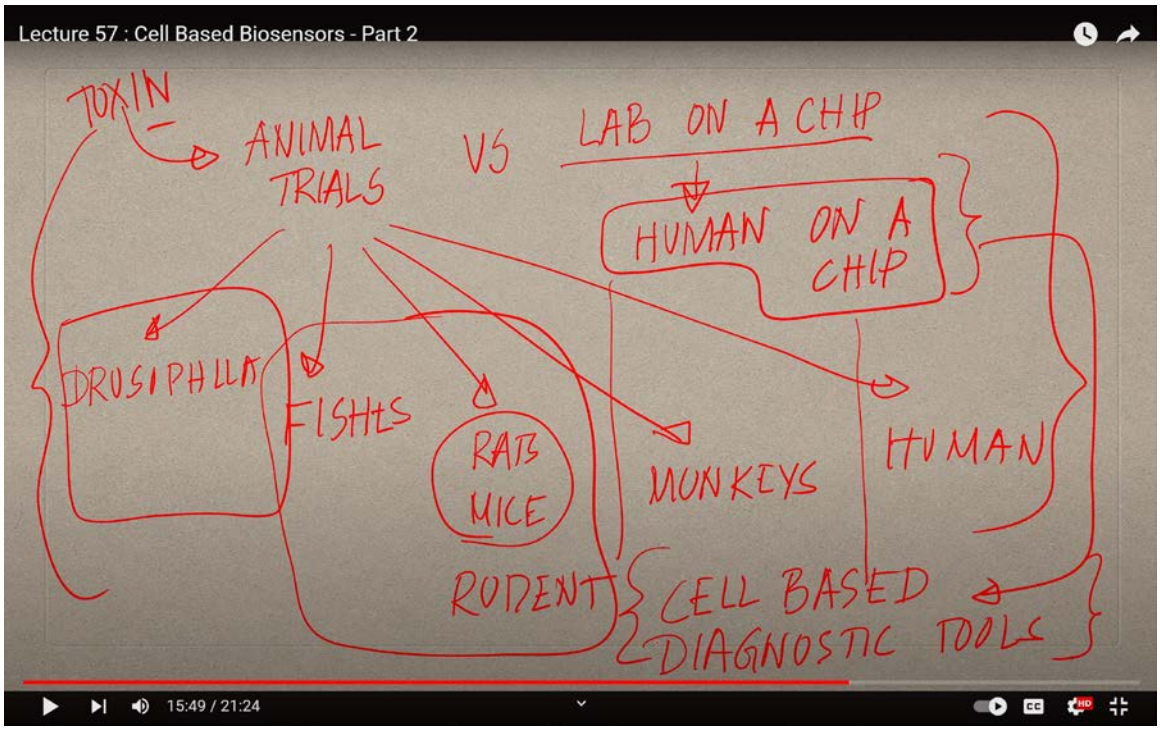

When we talk about planar microelectrode arrays, each array is typically designed with cells sitting on top of the electrodes, allowing us to detect the electrical activity from the cells. By adding toxins, metabolites, or any other compound, we can observe how the electrical activity changes over time.

The application of planar microelectrode arrays extends to fascinating areas. For instance, we know that our nervous system is highly organized, and traditionally, toxin testing has relied on animal trials. Animals such as Drosophila, fish, rats, mice, monkeys, and eventually humans are part of this testing paradigm. While I'm omitting many species here, the core point is that animal testing, especially for humans and primates, raises ethical concerns due to the cruelty involved. Testing toxins and viruses in animals for long-term effects is both ethically and technically challenging.

In recent years, however, there's been a significant shift toward reducing reliance on animal trials, and one promising alternative is the concept of a "lab on a chip" or a "human on a chip" system. This emerging technology is revolutionizing biosensor research and diagnostics, offering a more humane and effective way to study the effects of toxins and drugs. Unlike previous approaches, this technology represents a completely new class of development in biosensors, and I will provide several examples to demonstrate its potential.

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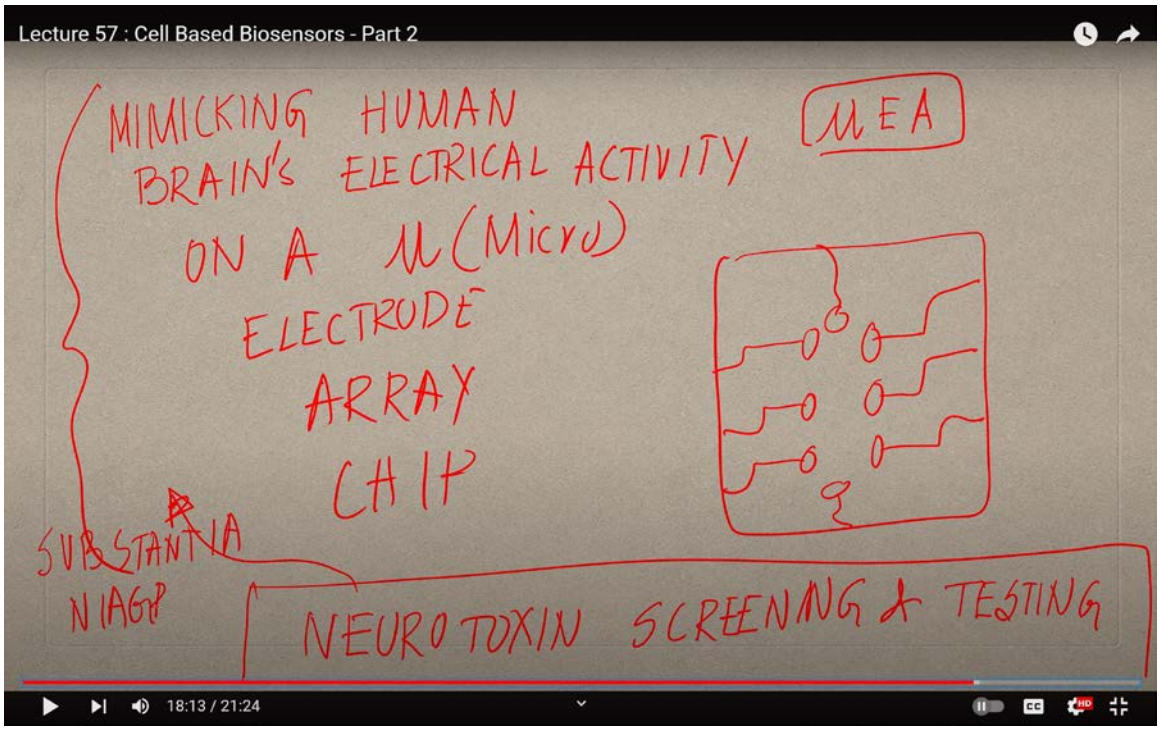

Returning to the microelectrode array, as I mentioned earlier, these arrays typically contain anywhere from 60 to 120 electrodes, making them electrically active chips. One of the primary areas where these arrays are being developed is for neurotoxin testing. The ability to test neurotoxins on these chips is particularly important for understanding how different compounds affect the nervous system without the need for animal testing, ultimately making the process more ethical and efficient.

Neurotoxin screening and testing can now mimic human brain electrical activity using microelectrode array chips. This is the first example of such studies that I will share with you. Imagine there's a neurotoxin that affects a specific part of the brain, such as the area responsible for movement. For instance, damage to the substantia nigra, a region rich in dopaminergic neurons, leads to conditions like Parkinson's disease. The substantia nigra is a critical area of the brain. If we visualize the human brain, it is a complex structure, with the spinal cord extending from it and various regions constantly interacting with one another. Each area, like those involved in learning, memory, or sensory processing, communicates dynamically with others.

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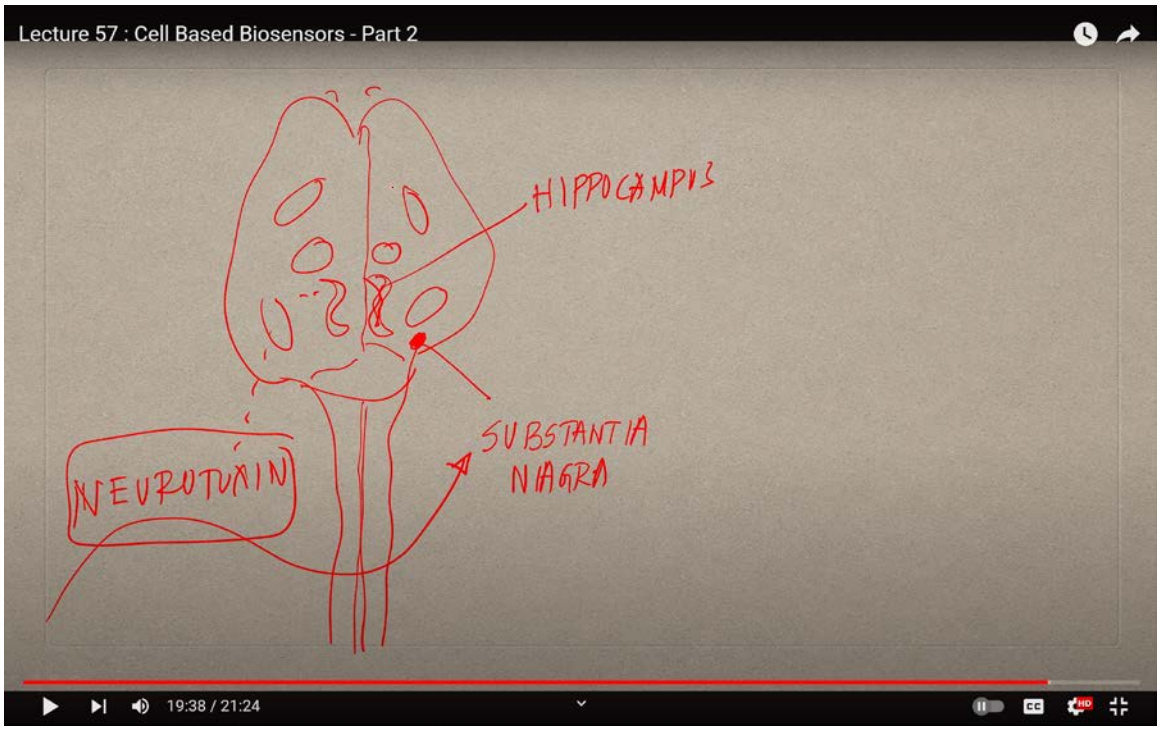

Now, say we focus on the substantia nigra. We know of a neurotoxin that specifically targets this area. What we do not yet fully understand is how this neurotoxin might influence other regions of the brain, such as the hippocampus (responsible for memory) or the visual cortex. This is where the power of mimicking human brain electrical activity on a microelectrode array chip comes in. Using this technology, we can explore how the neurotoxin behaves not just in the target region but also in other interconnected areas. We

will go into more detail about this process in the next class, where I'll explain how neurotoxin screening and testing are conducted on these chips.

In the upcoming class, I will also introduce how advancements in modern chip fabrication technology are revolutionizing biosensors. The next generation of biosensors is becoming increasingly sophisticated, integrating cell biology with photolithographic techniques and intricate patterning. These technological innovations are paving the way for more robust and accurate biosensors. So, in the next session, we'll dive deeper into how we can replicate the brain's structure and electrical activity. Thank you, and we will continue from here in the next class.