Design for Biosecurity

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Lecture 55

Action Potential - Part 5

Welcome back to our fifth class, where we will continue our exploration of various electrode configurations. In our last session, we discussed the first configuration: the intracellular setup. In this configuration, your electrode is positioned inside the cell, measuring the voltage and current, primarily focusing on voltage. Essentially, you are performing current clamp measurements.

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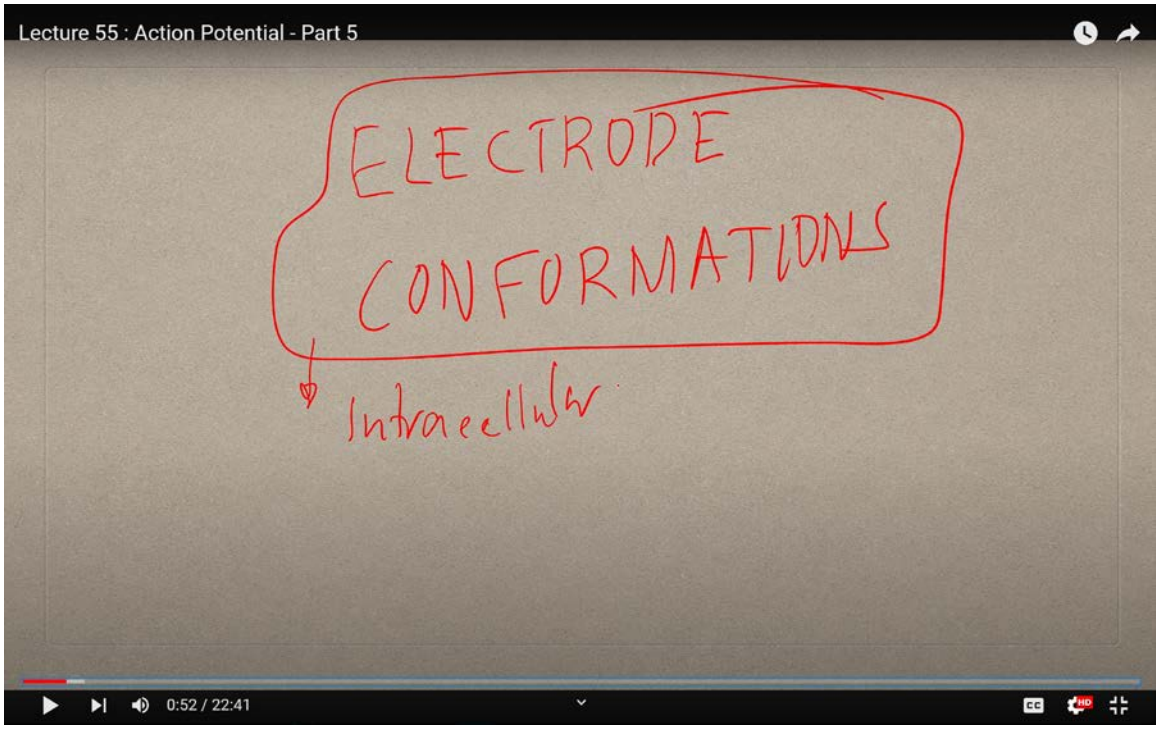

Now, let's transition to the patch clamp configuration. The necessity for patch clamp technology arose from a significant challenge. Whenever an electrode is inserted into a cell, leakage can occur, leading to rapid cell death. To address this issue, researchers

developed a method to minimize this leakage. Picture this: here’s the cell's surface, and you bring a glass electrode filled with either extracellular or intracellular fluid, depending on the configuration you're using.

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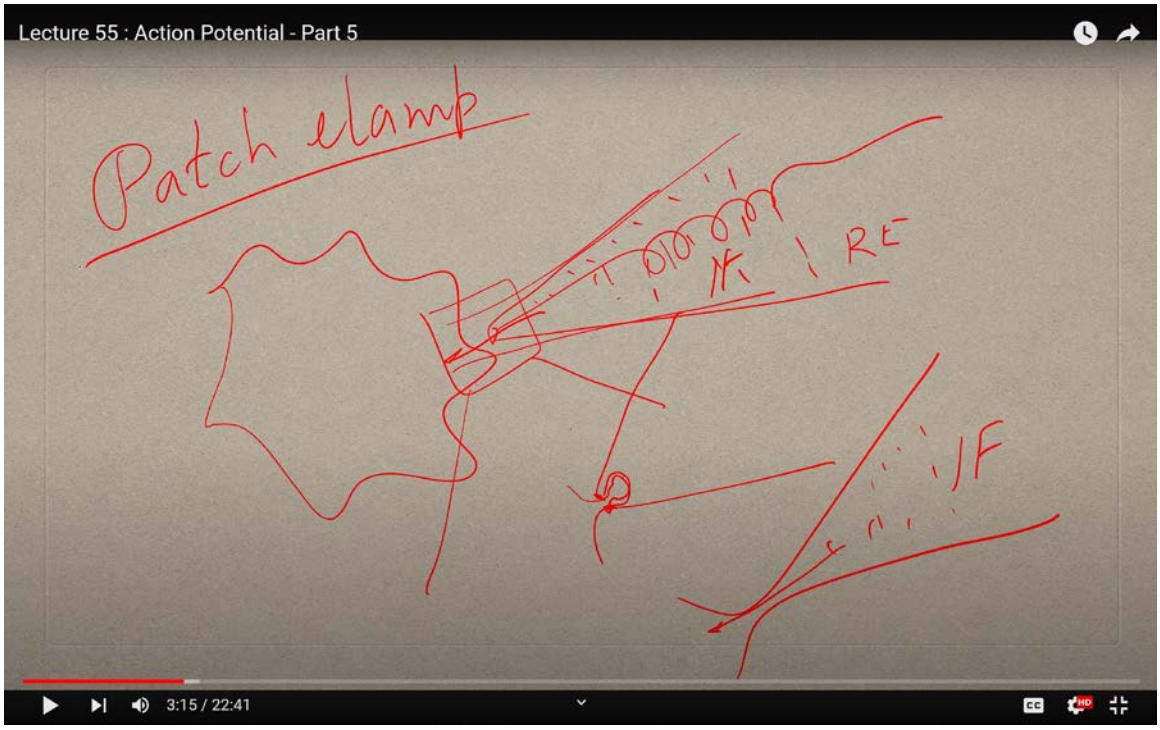

This is what we refer to as the recording electrode (Re). As you bring it closer, let’s magnify this part to see precisely what is happening at the membrane. By applying gentle suction, you can draw the membrane into the electrode. This critical advancement was primarily the work of Irwin Neher and Bert Sakmann. Eventually, what occurs is that you create a tight seal with the membrane, allowing it to become almost an integral part of the electrode.

If the electrode is filled with intracellular fluid (IF), it establishes continuity with the cell interior, enabling you to inject current into the cell effortlessly. You can alter the voltage and conduct various studies. While I won’t delve too deeply into the specifics of this discovery right now, I will share as much as I can. This configuration represents a groundbreaking achievement in electrophysiology, known as the patch clamp.

So, why do we call it a patch clamp? Because you are clamping, holding a specific section of the membrane, a patch, if you will. At this point, you gain direct access to the myriad of ion channels present in that localized area of the membrane. Often, researchers fill the electrode with extracellular fluid and then pull the membrane into the electrode, resulting in a configuration that resembles this: the tip of the electrode is positioned over the membrane.

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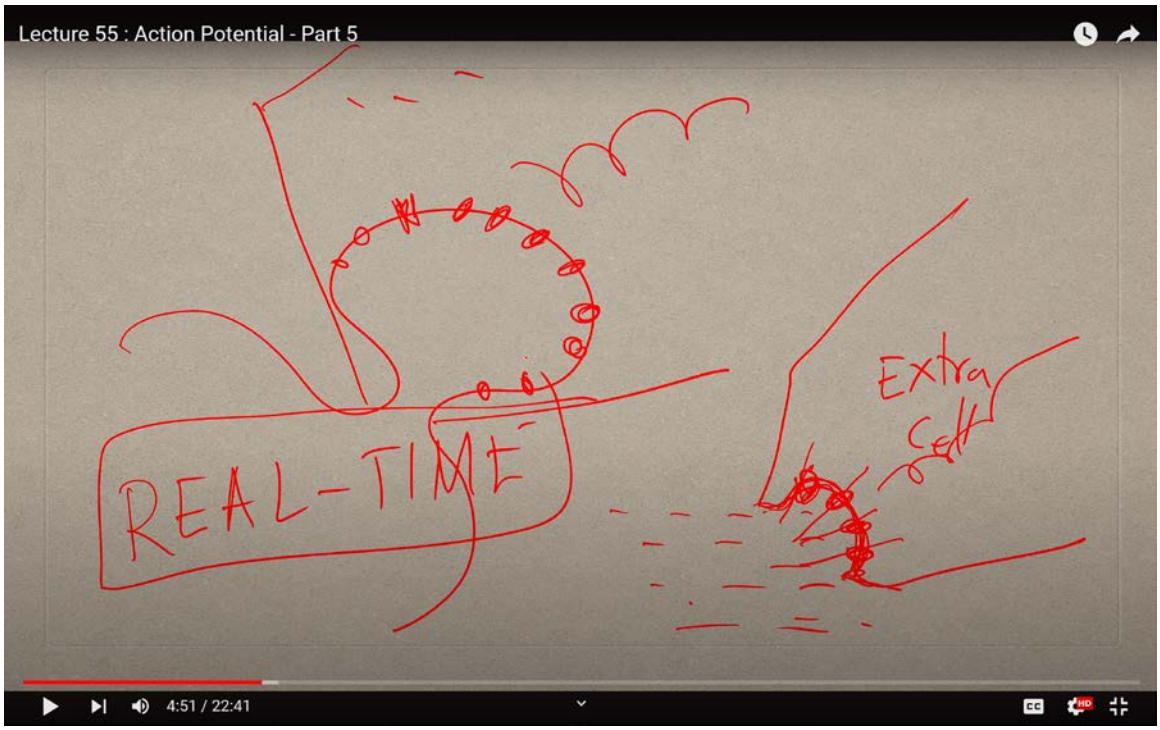

In this setup, you have part of the membrane incorporated within the electrode while the surrounding area is filled with extracellular fluid. When you dip the electrode into intracellular fluid, you effectively isolate the portion of the membrane under the electrode, allowing for precise measurements and manipulations. This innovative technique has truly revolutionized our understanding of cellular activity!

This technique is incredibly powerful because it allows us to observe the movement of molecules across proteins and membranes in real-time, almost as if the detached cells are unaware of their own separation. When I say "real-time," I mean it in contrast to other

techniques such as crystallography, cryo-electron tomography, and cryo-microscopy, which essentially freeze moments in time, capturing snapshots of biological processes. Electrophysiology, on the other hand, provides a continuous view of ion and molecule movements across membranes. This dynamic observation is unparalleled in any other technique, which is precisely why researchers favor this approach. It enables them to detect minute currents, nanocurrents, picocurrents, and even femtocurrents, flowing through some of nature's tiniest machines.

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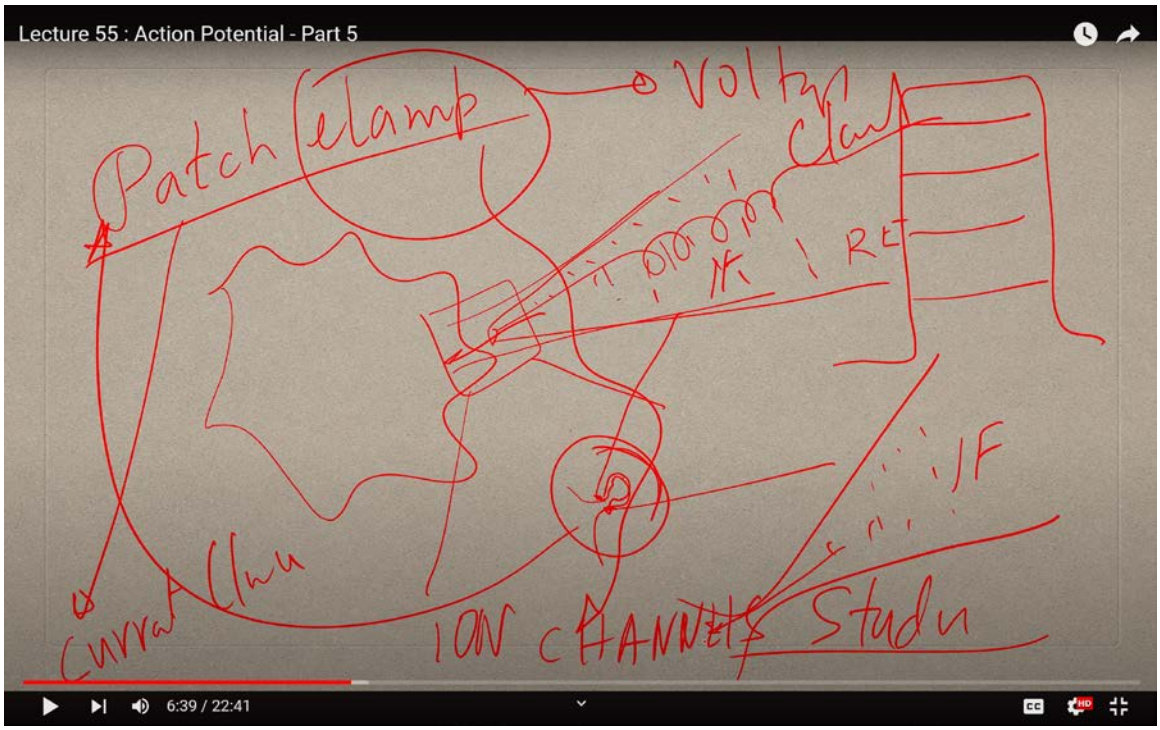

With this method, you can configure the system to interact closely with the cell, as I demonstrated in the previous configuration. You have the capability to vary voltage across the membrane with precision, applying both positive and negative voltage pulses. You can inject currents and monitor voltage changes in real-time, and vice versa. You can hold the voltage steady and measure the resultant current. The possibilities are virtually limitless! This is why voltage clamp techniques are often used in conjunction with current clamp techniques, although their primary focus is typically on voltage clamping.

Furthermore, this method paved the way for extensive studies of ion channels. One exciting development stemming from this is the planar patch clamp technique. While we've already discussed intracellular recordings and patch clamp configurations, let’s delve into the specifics of planar patch clamp recordings now.

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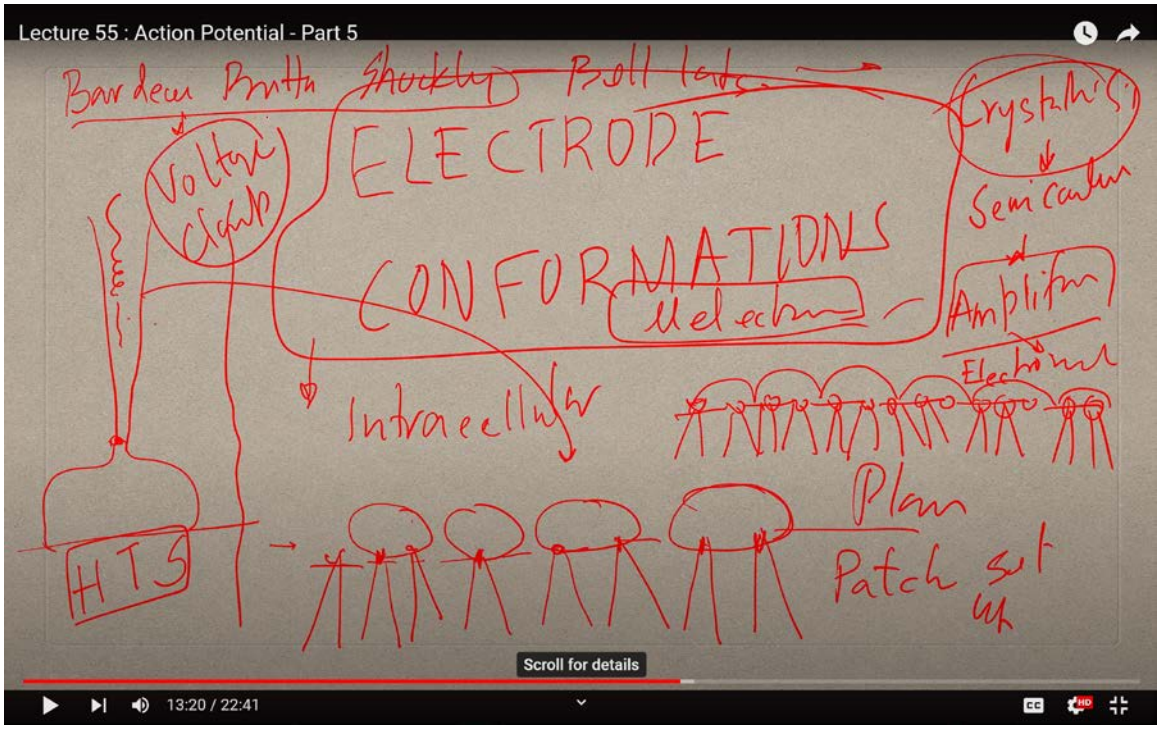

To illustrate this, let's refer to a slide. Imagine we are moving an electrode into position. While I won’t dive deep into the instrumentation details just yet, it’s worth noting that the patch clamp discovery and electrophysiological recordings gained significant momentum in the 1960s. This period was transformative, largely due to advancements in electronics in the 1940s and 50s. During this time, three brilliant scientists, William Shockley, John Bardeen, and Walter Brattain, who were all affiliated with Bell Labs, made groundbreaking discoveries in crystalline silicon and its semiconductor properties. Their work revolutionized the microelectronics field, enabling the development of powerful amplifiers capable of detecting signals from extremely low current ranges.

As a result of these advancements, highly sensitive electrometers were developed, thanks to the properties of crystalline silicon as a device material. This marked the dawn of the microelectronics era. Notably, Bardeen, Brattain, and Shockley were awarded the Nobel Prize for their pioneering work, with Bardeen later receiving a second Nobel Prize for his contributions to semiconductor theory.

The biosensor field was significantly influenced by the microelectronics revolution, transforming how researchers approached biosensing and measuring lower currents. During this time, Irwin Neher and Bert Sakmann capitalized on these advanced amplifiers. It's important to acknowledge that many of the innovators behind these amplifiers may not have received the recognition they deserved, yet their contributions fundamentally altered the landscape of biosensing for the better!

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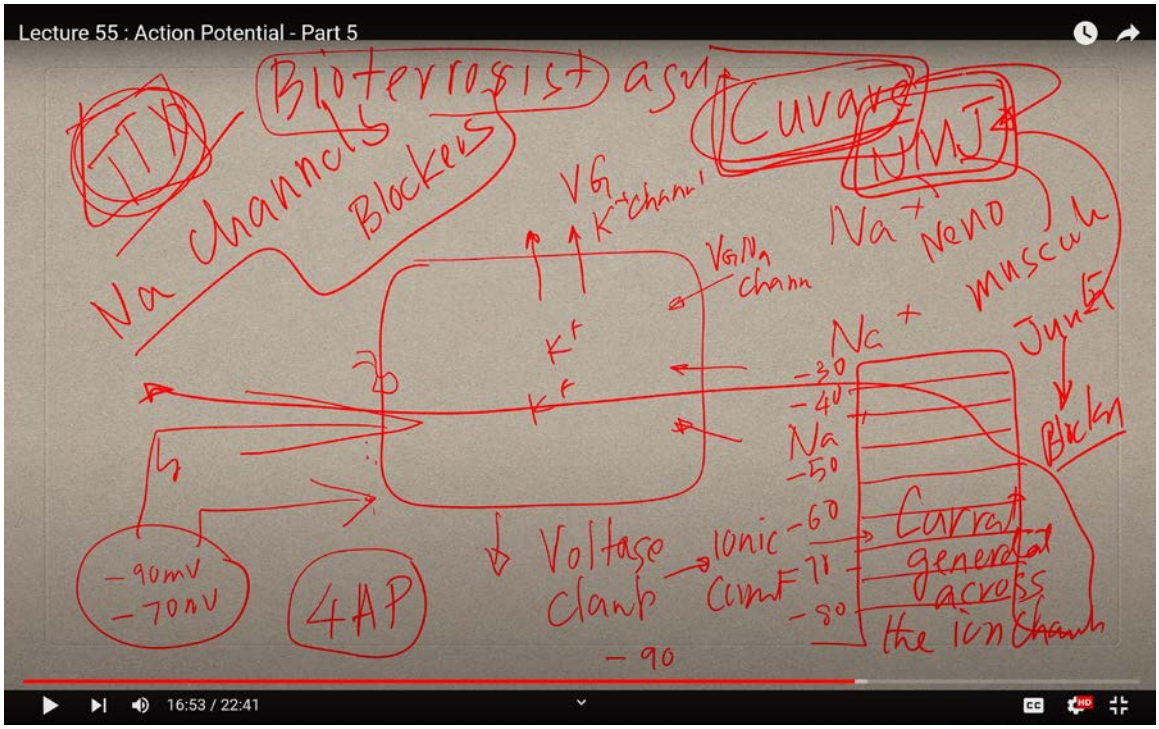

This marks a pivotal moment when a plethora of electronics began to infiltrate the biological realm for recording and various other purposes. One of the most significant advancements during this period was the emergence of patch clamp technology, which

harnesses some of the most powerful amplifiers developed by companies like HEKA and others in the field.

Now, as we delve into the patch clamp technique, envision cells resting on a dish, where researchers meticulously manipulate a section of the membrane to conduct their studies. However, in the 1990s, this process proved to be rather tedious, prompting scientists to innovate a new method. Instead of creating a hole in the membrane, they devised a surface where cells could grow over nanopores. These nanopores are essentially small holes, beneath which electrodes are strategically positioned. This innovative approach eliminates the need for manual manipulation; all that's required is to deliver pulses that will break the membranes and establish a planar patched setup.

In this configuration, the cells sit atop the electrodes, rather than approaching them from above. This means that cells grow directly over the embedded electrodes on the surface. The process allows for the possibility of targeting one cell with multiple electrodes simultaneously, which, while feasible, can still be quite labor-intensive. By controlling the surface environment, researchers can achieve exceptionally high-quality recordings.

Although this discovery is relatively new and not yet widely recognized in high-throughput screening (HTS) for drugs and toxins, it has gained considerable traction over the past two decades. If memory serves me right, I first encountered evidence of this linear patch-clamp array at a neuroscience congress in San Diego, either in 2001 or 2002. I felt fortunate to witness this technology firsthand; it was remarkable to observe how it streamlined the recording process.

Since that time, I've continued to monitor its development within the industry. However, it's important to note that while the technology has advanced significantly, challenges remain. The visual representation I'm providing on these screens is not just a simple computer display; there have been simultaneous developments that enhance these recordings.

Essentially, when we discuss patch clamp technology, we're emphasizing its primary advantage, voltage clamping. You might wonder why understanding these currents is so

crucial. If we revisit our earlier discussions about various toxins, such as 4-aminopyridine, curare, and tetrodotoxin (TTX), it becomes clear that these substances are not broadly impactful. Instead, they are highly specific, often binding selectively to sodium channels or targeting only certain portions of those channels.

These toxins have the ability to bind to potassium channels or even selectively attach to specific portions of those channels, leading to a reduction in sodium current. Notably, many of these bioterrorism agents exhibit either invasive characteristics, which can completely devastate the cell membrane, rendering it non-functional, or they take a more insidious approach. These sophisticated toxins specifically target parts of sodium channels, gradually weakening the body without the victim's awareness. This highlights the critical importance of having direct access to voltage clamps, enabling us to study the ionic currents, also known as the currents generated across the ion channels.

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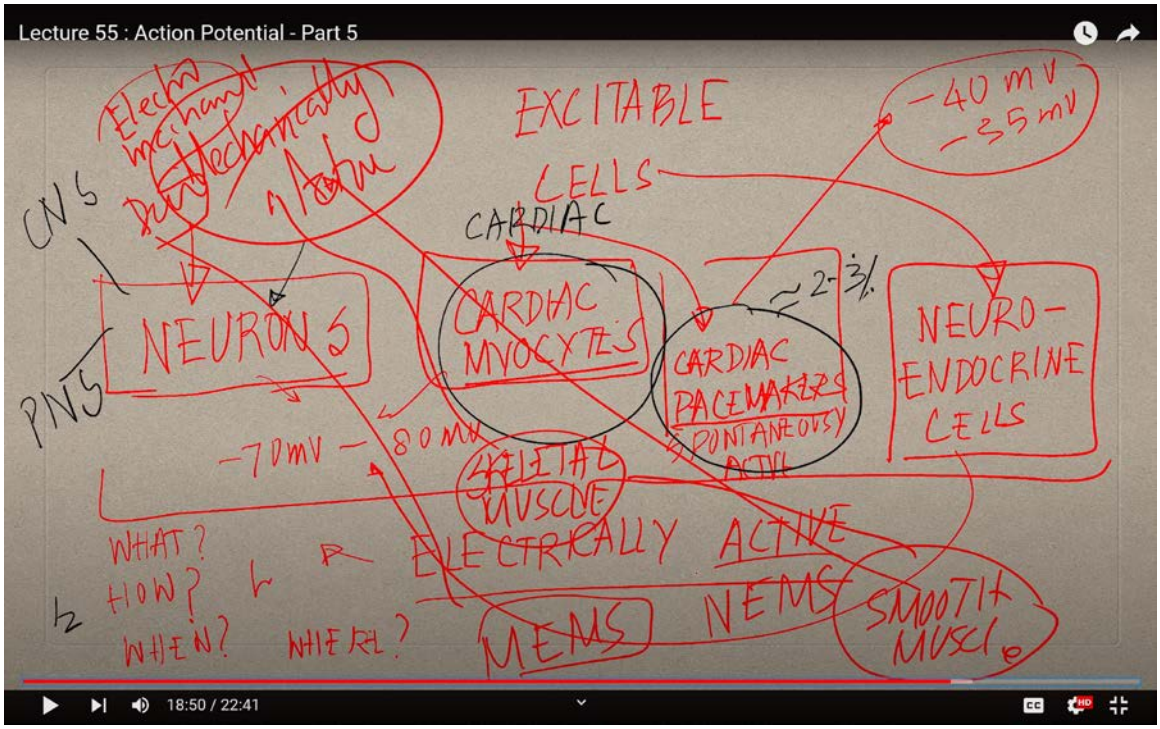

These ion channels operate at an incredibly small scale, on the order of angstroms. It’s astonishing to realize that these are some of the tiniest machines humanity has the privilege

to observe today. We are living in an extraordinary era, where we can visualize the architecture of these remarkable structures, at resolutions close to 3.5 angstroms. The potential applications of such machines are vast; for instance, they could revolutionize the process of filtering seawater. Imagine having a channel that selectively allows sodium to pass through, if we could emulate such channels and structures, we could fundamentally alter our approach to the global water crisis. With oceans being the largest water source, developing effective purification methods is essential.

While we discuss the threat of bioterrorism, it's crucial to consider the countermeasures we are developing. All these concepts converge, illuminating the remarkable insights we are gradually uncovering. For example, toxins like curare, prevalent in various tribal practices, specifically target the neuromuscular junction, disrupting the transmission of electrical impulses from nerves to muscles.

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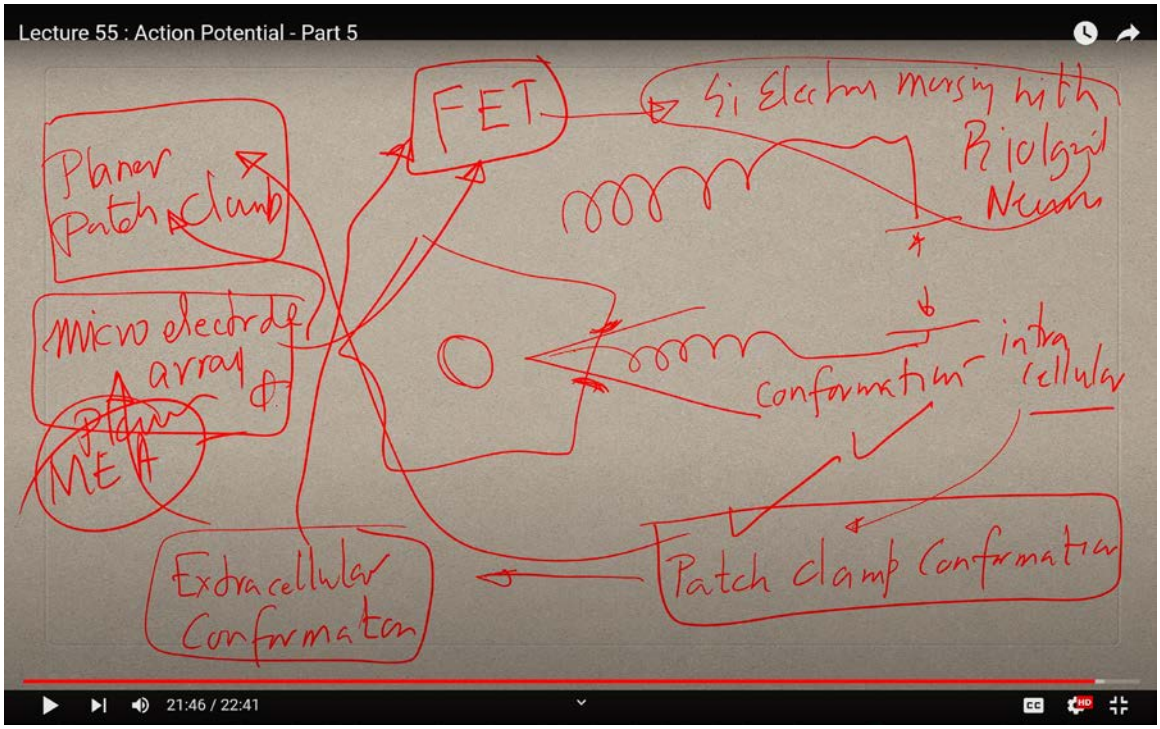

There's one more category of excitable cells I haven't mentioned yet, and I'd like to take this opportunity to discuss neurons, alongside skeletal and smooth muscle cells. These are

not only excitable; they also exhibit mechanical activity. In fact, these cells possess a contraction coupling apparatus that enables movement, your hands can wave, and your heart can beat. Essentially, they function as electro-mechanical devices, akin to what engineers refer to as micro-electro-mechanical systems (MEMS).

In nature, we have what can be described as nano-electro-mechanical systems (NEMS). This is a testament to how evolution has developed these intricate systems. While we are currently taking a top-down approach to understand them, nature has already created NEMS and MEMS long before our technological advancements. These systems span the disciplines of mechanical engineering, microelectronics, material science, and biology. Nature has already exhibited constructs like nanoelectromechanical systems, exemplified by cardiomyocytes, skeletal muscle cells, cardiac pacemaker cells, and smooth muscle cells. The smooth muscles, which facilitate various bodily movements, represent some of the most refined machines we have the privilege to study.

Now, let me explain why this understanding is so crucial. By comprehending the ionic currents, we can discern the precise action of every toxin, unlocking the beauty of the whole cell biosensor field. This technology has vast applications: we can employ these sensors for insecticides, pesticides, and beyond. The opportunities are staggering, opening up a world of possibilities that are truly beyond imagination.

As I mentioned earlier, we'll transition from discussing planar patch clamps to exploring extracellular conformations, followed by microelectrode arrays. Then, we’ll delve into the fascinating realm where silicon electronics converge with biological neurons. This area encompasses the exciting frontier of field-effect transistors (FETs), merging solid-state electronics with electrochemical sensing. This innovative marriage allows us to achieve recordings that, while dealing with ionic motions, which are considerably bulkier, can still be extremely noisy. It's important to note that this field has yet to gain widespread popularity.

Germany played a pivotal role in the initial breakthroughs of many of these technologies, making it a significant hub for research and innovation. There is still so much to understand and explore in this domain.

In our next class, we will begin with the extracellular conformations, focusing on how the recordings are performed. After that, we'll turn our attention to microelectrode arrays, often abbreviated as MEAs or planar MEAs, due to their structure. Lastly, we will explore the exciting world of field-effect transistors.

I will conclude today’s class here, and I eagerly look forward to continuing our journey through the fascinating developments in biosensors, their applications, and their role in shaping our countermeasures against bioterrorism. Thank you!