Design for Biosecurity

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Lecture 56

Cell Based Biosensors - Part 1

Welcome back to this class on design for biosecurity. In the last session, we delved into the patch clamp technique, and I explained the concepts behind current and voltage clamping. Let me quickly recap: when we talk about the current clamp, we are fixing the current and measuring the voltage. This is typically used when measuring action potentials. On the other hand, in a voltage clamp, you clamp the voltage and measure the current. I also explained how we can manipulate the voltage in these setups.

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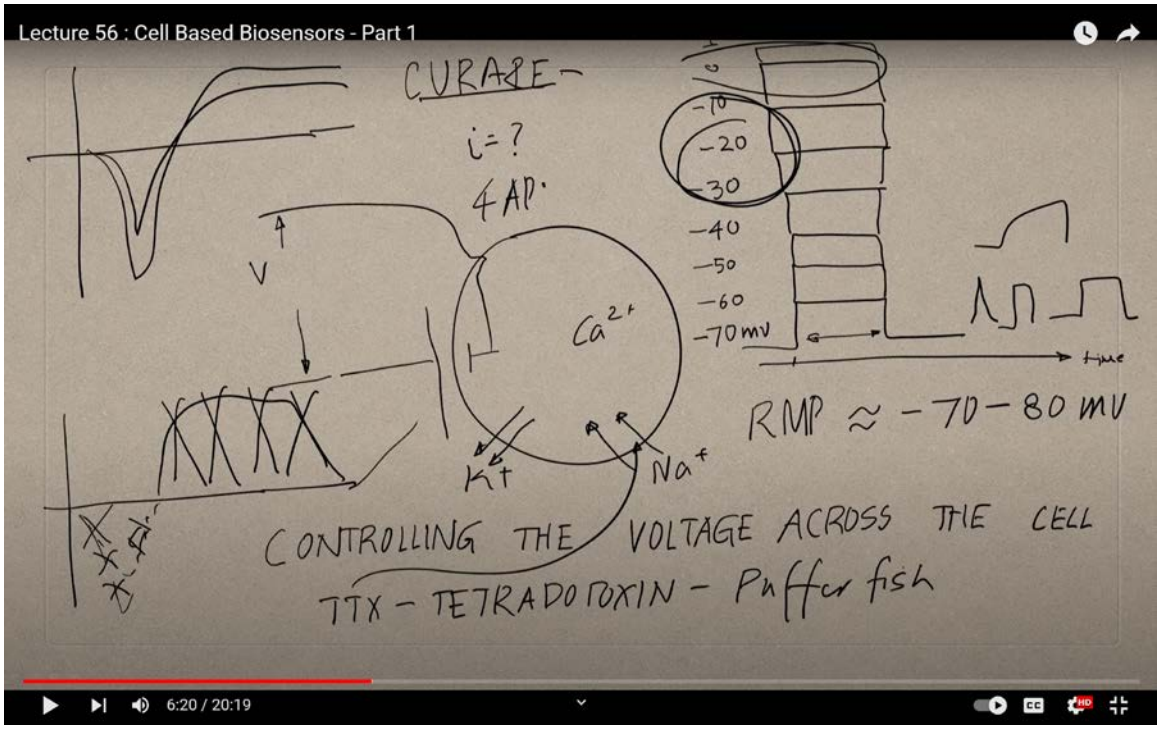

Imagine a cell where we know the inside is negatively charged, and the outside is positively charged, like a battery. By controlling the voltage across the cell, you can dictate the

behavior of ion channels, i.e., their opening and closing. Essentially, you're creating a situation where you control the voltage step-by-step. For instance, we know the resting membrane potential is around -70 to -80 millivolts. You could design a protocol where you increase this voltage in increments of 10 millivolts. So, from -70 millivolts, you could go to -60, -50, -40, and so on, all the way to 0 or beyond. The time each pulse lasts is something you decide, which is usually based on empirical knowledge passed down through years of research. However, you always have the freedom to adjust the parameters, whether it's using shorter or longer pulses or changing the pulse shape entirely. You can experiment with countless variations.

Now, while you manipulate the voltage, you measure the current. If you recall, I mentioned that you'd typically see an inward sodium current followed by an outward potassium current. This inward sodium current corresponds to the opening of sodium ion channels, and the potassium current corresponds to the opening of potassium channels. In essence, you are observing the movement of sodium ions into the cell and potassium ions out of it.

This technique isn't limited to just sodium and potassium; you can even apply it to calcium, though that's a bit trickier. For instance, imagine a bioterrorist agent like tetrodotoxin (TTX), which comes from pufferfish. TTX blocks sodium channels. So, if you were performing a voltage clamp in the presence of TTX, you wouldn't see any sodium currents, those would be completely absent. Instead, you'd only see the potassium current. In this way, you can isolate different currents using specific toxins. For example, if you used 4-aminopyridine (4-AP), a potassium channel blocker, you would see the potassium current disappear.

Another example is curare, a toxin traditionally used by tribes in the Amazon basin. Curare affects the motor neurons that control muscles. We'll talk more about this in the next slide, but essentially, it targets the connection between motor neurons and muscles, disrupting their function.

So, to sum up, through these voltage and current clamp techniques, we can control and observe the intricate dynamics of ion flow, manipulate them using toxins, and ultimately

gain insight into how different agents, whether bioterrorist or therapeutic, affect cellular function.

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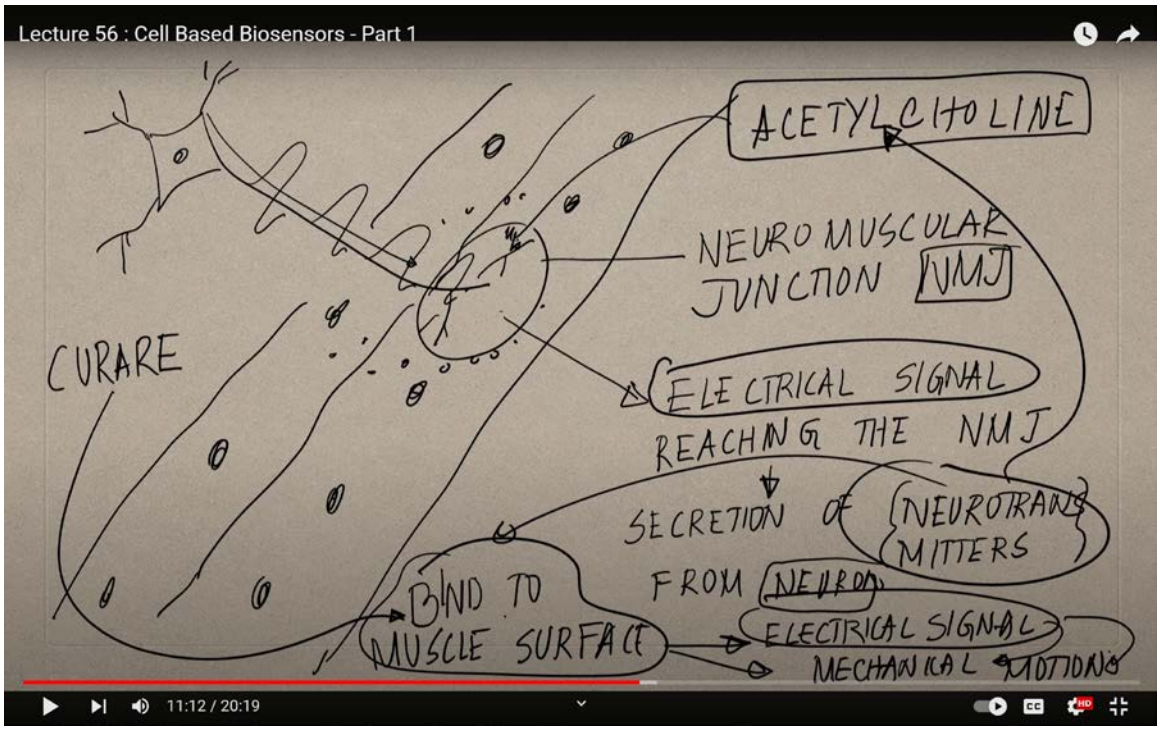

Now, let's focus on what happens at the neuromuscular junction (NMJ), a crucial spot in the body where nerves and muscles communicate. This junction is extremely important from multiple perspectives, particularly if you're trying to target a drug or toxin to induce paralysis. Historically, when humans relied on hunting, they discovered various toxins from sources like plants, fungi, and snake venom. Many of these toxins were specifically designed to paralyze animals by acting directly on the neuromuscular junction.

Understanding how these toxins work requires first understanding what happens at the NMJ. The process begins when an action potential (an electrical signal) travels along a motor neuron and reaches the neuromuscular junction. This is step one: the electrical signal arrives. Step two involves the secretion of neurotransmitters from the neuron, which then bind to the surface of the muscle. This binding initiates two simultaneous actions. First, an electrical signal is triggered in the muscle, and second, this electrical signal sets off

mechanical motion in the muscle. The process is a complex orchestra where an electrical signal transforms into a chemical signal, which then reverts back to an electrical signal, ultimately leading to a mechanical action.

This entire chain of events is precisely coordinated, and it can only be disrupted by chemically interfering with the process. For instance, a toxin like curare can disrupt this process. When curare is introduced near the neuromuscular junction, it binds to the same receptors on the muscle surface that neurotransmitters (such as acetylcholine) are supposed to bind to. By occupying these binding sites, curare prevents acetylcholine from activating the muscle.

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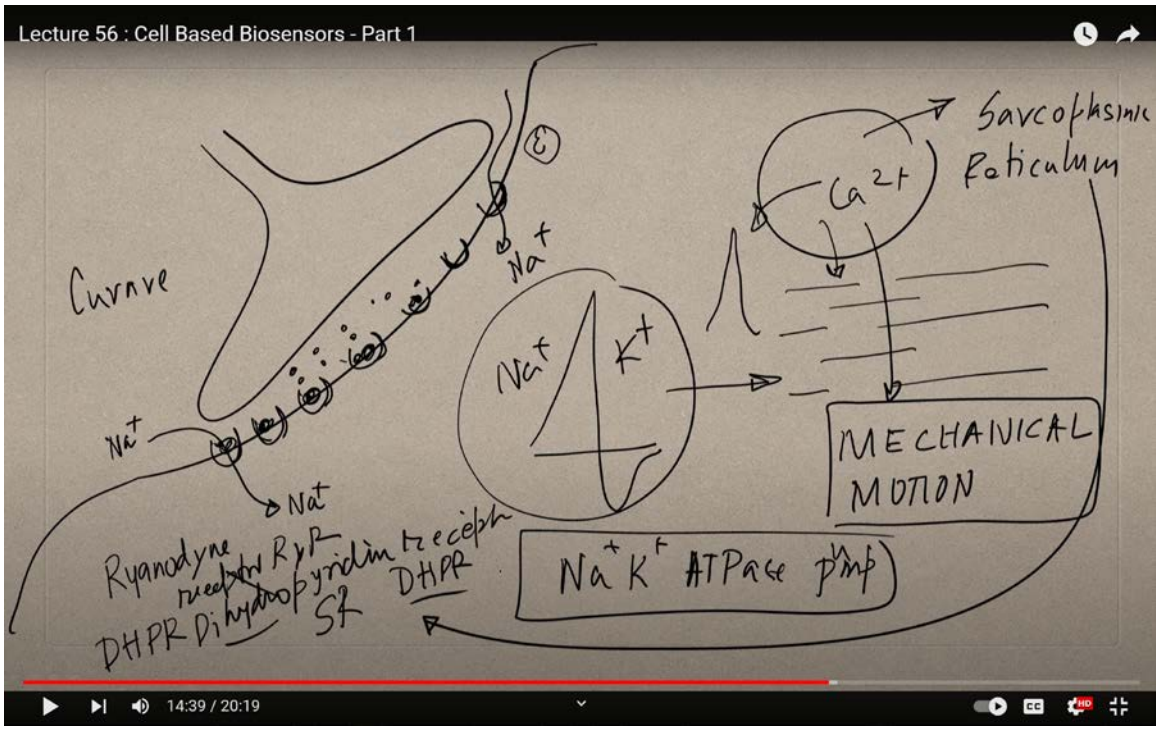

To explain in more detail, acetylcholine is the primary neurotransmitter at the NMJ, and once it is released from the neuron, it binds to acetylcholine receptors on the muscle surface. This binding opens up cation channels, allowing sodium ions to flow into the muscle. This influx of sodium generates another action potential within the muscle, which sets off a series of events inside the muscle fibers. These events involve the interaction

between actin and myosin filaments, leading to muscle contraction, what we recognize as muscle movement. Calcium plays a vital role in this process, as calcium currents are necessary to drive these contractions, along with sodium and potassium currents and the activity of the sodium-potassium ATPase pump.

Chemically, you can block this transmission at various stages. With curare, for instance, the toxin binds to the acetylcholine receptors, effectively blocking the sodium influx needed to generate an action potential. Without this sodium influx, the muscle doesn't receive the signal to contract, leading to paralysis.

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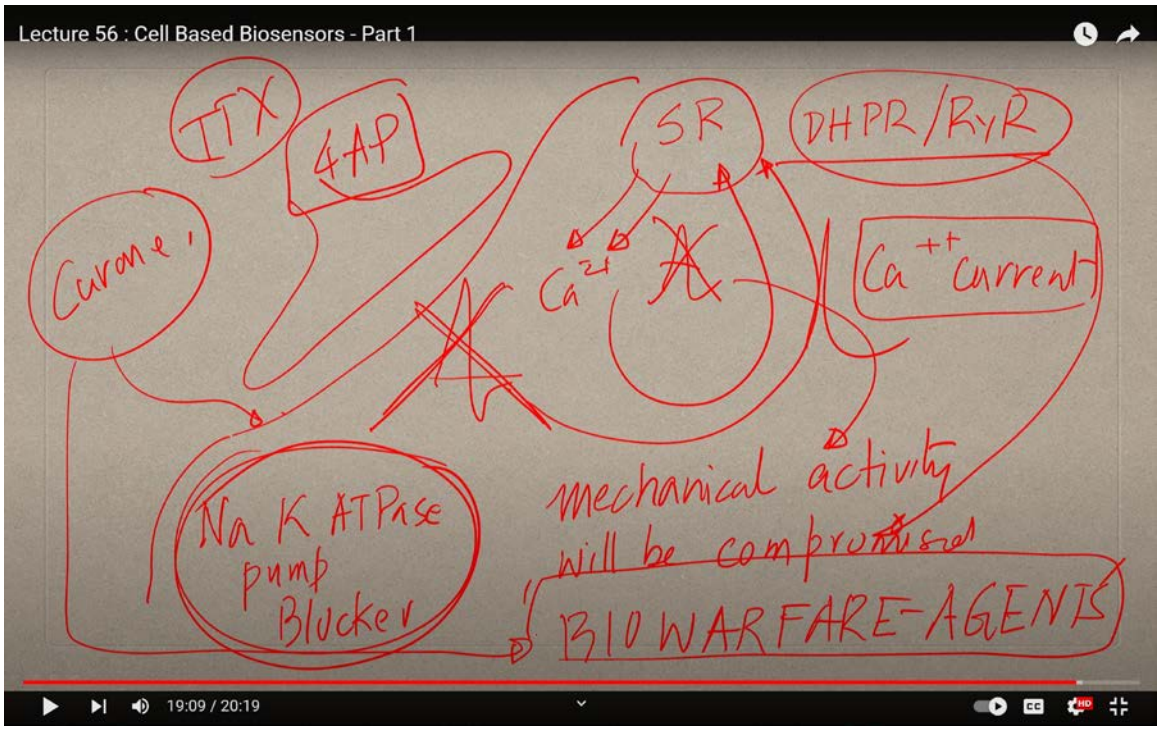

But that's not the only way to disrupt the process. Calcium blockers can also stop muscle movement by targeting another crucial organelle in the muscle called the sarcoplasmic reticulum (SR). The SR contains two key calcium channels: the ryanodine receptor (RYR) and the dihydropyridine receptor (DHPR). These receptors work in tandem, releasing calcium when needed and then quickly pulling it back into the SR. Calcium is essential for

muscle contraction, so if a toxin blocks these calcium channels, mechanical motion is halted because the muscle doesn’t receive the calcium spike required to contract.

Thus, the neuromuscular junction is a highly orchestrated system where electrical, chemical, and mechanical signals work in harmony. Toxins like curare and calcium blockers can disrupt this process at multiple levels, whether by preventing the action potential, blocking calcium release, or inhibiting sodium influx, ultimately leading to paralysis. The sarcoplasmic reticulum, with its calcium-handling machinery, plays a particularly fascinating role in this mechanism.

The sarcoplasmic reticulum (SR) is an essential player in muscle function, as it regulates the flow of calcium. Calcium is released, or "effluxed," from the SR and then quickly pulled back by calcium pumps. This process is vital because the transient increase and decrease in calcium concentration, known as the calcium current, is tightly controlled to prevent damage to the cell. Calcium, if not regulated properly, can become excitotoxic, meaning it can be harmful or even lethal to the cell.

To release calcium, two main players are involved: the dihydropyridine receptor (DHPR) and the ryanodine receptor (RYR). These receptors facilitate a quick burst of calcium, followed by a rapid retraction, creating the calcium current. However, if you introduce DHPR or RYR blockers, or certain toxins, this entire process can be halted. As a result, the mechanical activity of the muscle is compromised, which means the muscle loses its ability to contract properly.

There’s also another way to affect this process: by targeting the sodium-potassium ATPase pump. If you use a blocker for this pump, you can disrupt the ion balance required for action potential generation and muscle contraction. So, there are multiple ways to interfere with these events.

To summarize, there are several pathways through which muscle activity can be blocked. For instance, you could use tetrodotoxin (TTX), which blocks sodium channels. When sodium channels are blocked, sodium currents can't form, preventing the generation of action potentials. Without action potentials, the SR can't release calcium, and without

calcium release, the muscle can't contract. The same principle applies when using curare, which blocks acetylcholine binding sites, stopping the action potential from being generated and halting all subsequent events.

Similarly, you could use toxins that block the sodium-potassium ATPase pump, further disrupting muscle function. These examples demonstrate how sensitive the human body is to disruptions, and how easily these essential processes can be interrupted. Toxins like TTX, curare, and 4-aminopyridine (4-AP) are highly regulated substances, often considered biowarfare agents. Possessing or using these compounds without proper authorization is illegal in many countries, much like owning a gun requires a permit.

You cannot simply buy these substances; you need explicit permission from relevant authorities to possess or work with them. The list of restricted compounds is extensive, and each is carefully controlled due to its potential use in biowarfare.

In the next class, we will explore some techniques to better understand how these compounds interact with the neuromuscular junction, including how the junction can be used as a sensor. We will also move forward with discussions on extracellular recordings and field-effect transistors. Thank you.