Design for Biosecurity

Prof. Mainak Das

Biotechnology and Bioengineering

Indian Institute of Technology, Kanpur

Lecture 54

Action Potential - Part 4

Welcome back to class! In our last session, we concluded with a discussion on the action potentials of neurons, and I promised to delve into the action potentials of cardiomyocytes today. When we examine cardiac cells, we notice something quite unique. Let's refer to the graph we've been drawing, with time measured in milliseconds on the x-axis and voltage in millivolts on the y-axis.

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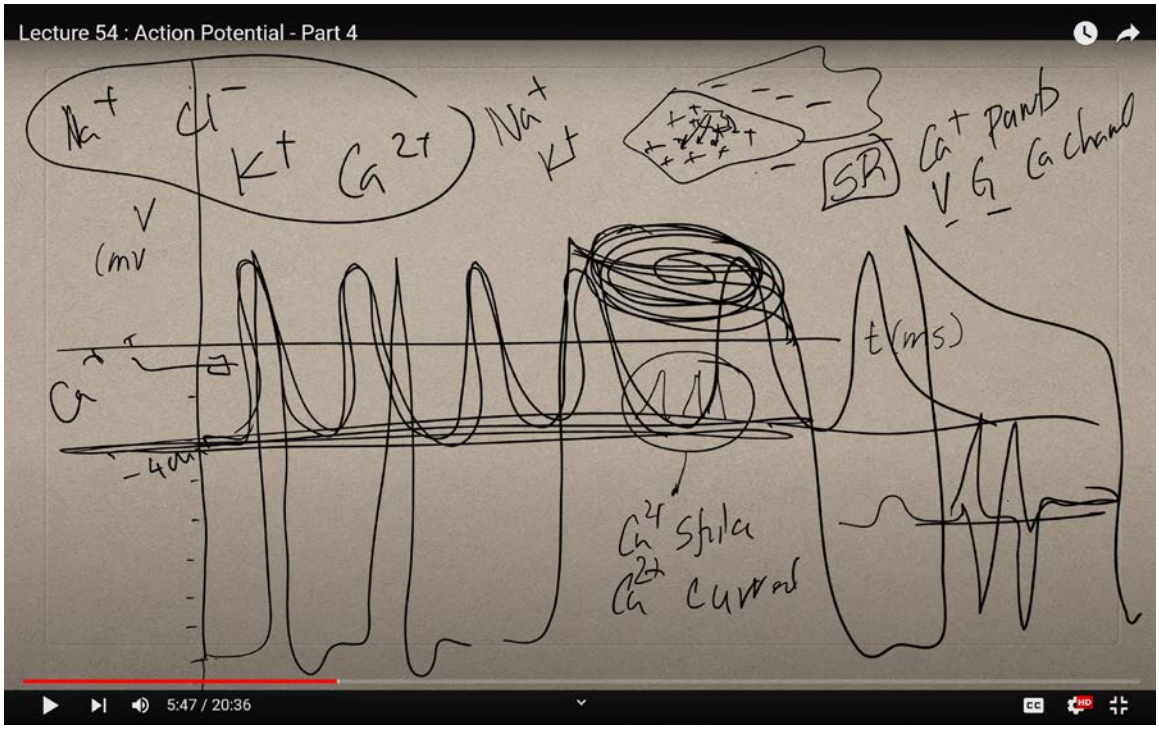

For neurons, the action potential typically appears as a rapid overshoot followed by a return to baseline. In contrast, the action potential of cardiomyocytes presents a different trend. The curve starts with an initial spike, remains elevated for a significant period, and then

gradually returns to baseline.

This distinctive plateau phase in the cardiac action potential can vary in shape; sometimes it may appear more pronounced, while at other times, it may be more subdued. What does this mean? Simply put, this plateau occurs due to a prolonged presence of cations within the cells. So, which cations are we talking about? We've already discussed sodium and potassium, but there's another crucial cation involved here, calcium.

Let's take a moment to revisit what we discussed in previous classes. I mentioned calcium previously, emphasizing that this ion must be carefully regulated within the cell. If calcium is not managed properly, it can become cytotoxic or lead to excitotoxicity, resulting in extreme hyperexcitability. Now, let's clarify the role calcium plays in the context of cardiomyocytes.

These cells possess a highly specialized structure known as the sarcoplasmic reticulum. While this structure is found in all muscle cells, it is particularly prominent in cardiomyocytes, equipped with numerous calcium pumps and voltage-gated calcium channels. During the action potential, cardiomyocytes momentarily release a substantial amount of calcium before quickly reabsorbing it. You can think of this process as similar to a sponge used for cleaning dishes. Just like how you squeeze a sponge to remove water and then let it soak up more, cardiomyocytes experience a surge of calcium ions followed by a rapid retraction.

This sudden influx of calcium creates what we refer to as a calcium spike or calcium current, which is crucial for establishing that plateau phase in the cardiac action potential. At this point, we can identify a fourth important component influencing the generation of action potentials: in addition to sodium, potassium, and chloride, calcium plays a pivotal role.

When we discuss pacemaker cells, it's important to note that they exhibit a different action potential pattern altogether. So, as we continue our exploration of cardiac physiology, let's keep these components in mind and delve deeper into how they interact to create the rhythmic contractions of the heart!

These pacemaker cells typically rest at around -40 millivolts, though the graph I'm showing reflects a voltage of -20 millivolts, just for illustrative purposes, let's assume it's actually -40 millivolts. When they reach this voltage, there is a significant surge in activity due to the presence of specialized calcium channels. It's important to note that sodium is not the primary player in this scenario; rather, calcium plays a crucial role in the generation of spontaneous action potentials and in the pacing of our heart. This process is what you observe in an electrocardiogram (EKG) trace, many of you may have seen these EKG patterns.

This is the essential foundation you need to grasp regarding the concept of action potentials. Now, let's move on to another aspect of excitability. I previously mentioned the various players involved, sodium, potassium, and more. Sodium enters the cells through voltage-gated sodium channels, while potassium exits via voltage-gated potassium channels. Additionally, we have pumps that regulate these movements.

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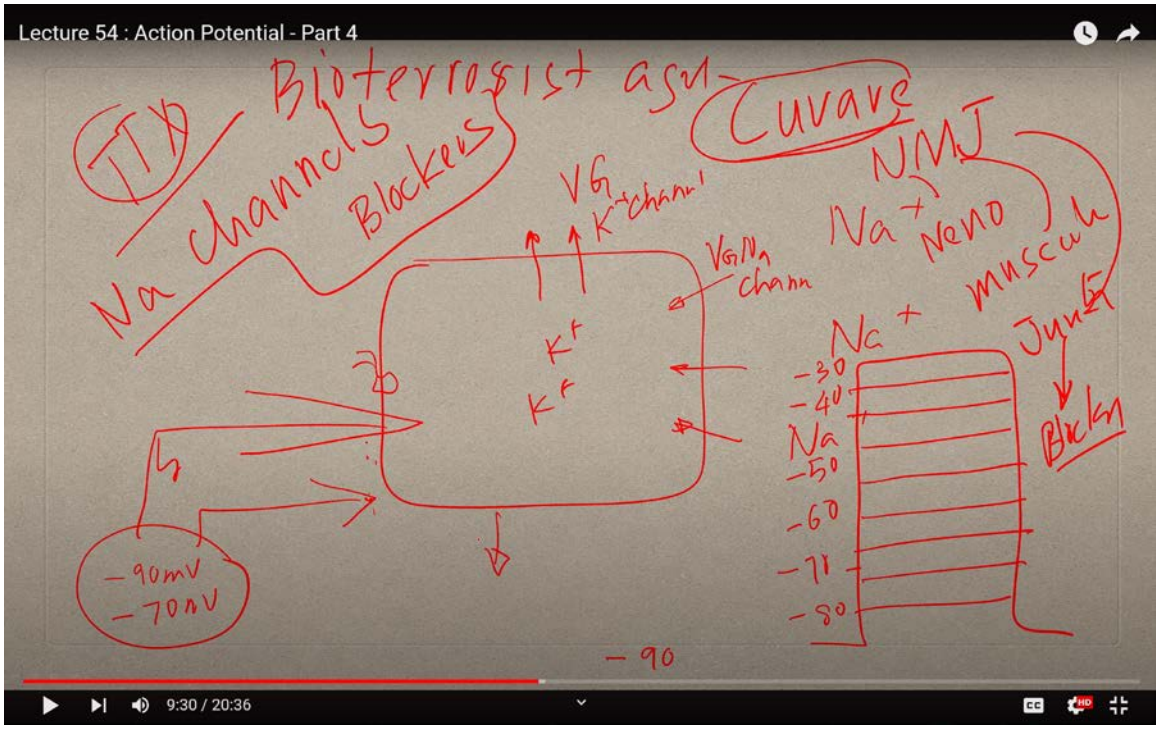

We know that the voltage difference across the membrane outside to inside typically sits

at about -90 or -70 millivolts. Here, we're discussing regular excitable cells, not just pacemaker cells. Now, let’s imagine a hypothetical scenario: if we could artificially manipulate a cell’s voltage through electronic means. Picture this: starting from -90 millivolts, you send a voltage pulse that progresses like this: -90, -80, -70, -60, -50, -40, and -30 millivolts. As these voltage pulses are applied, you can activate the ion channels, which respond based on the voltage across the membrane.

What’s fascinating here is that you can synthetically activate these channels that are typically triggered by ligands, substances that bind to them. These ligands can include photons, neurotransmitters like acetylcholine, or even toxins that influence the channels' states. One such toxin is tetrodotoxin (TTX), a potent sodium channel blocker often associated with bioterrorism. Sodium channel blockers like TTX represent a significant risk, as they can incapacitate a population by interfering with vital physiological functions.

Similarly, consider curare, which acts at the neuromuscular junction (NMJ), where the "N" stands for neuro, "M" for muscular, and "J" for junction. These neuromuscular junction blockers are also classified as potential bioterrorism agents. The ability to paralyze an entire population using such toxins underscores their danger.

This leads us to a compelling aspect of biosensing. As I mentioned earlier, you can study these ion channels by varying the voltage applied across the membrane. This method provides a fascinating avenue for understanding and potentially harnessing these channels for various applications in bioelectronics and biosensors!

For those of you with a background in molecular biology, you may be aware that it is possible to express these ion channels using molecular biology tools in significantly larger quantities. You could create cells that exclusively contain sodium channels or cells that only have potassium channels by manipulating the DNA and expressing these channels in different species or cell types. This allows you to study these channels extensively, as they serve as the real sensor elements in cellular function.

Now, let’s discuss how we can measure these currents during the action potential, which is characterized by a specific shape. In this graph, we have a baseline at 0 volts, with the -

80 millivolt line marked below. The action potential event involves three different currents: the potassium current, the sodium current, and, of course, the contribution from the pumps. But how exactly can we measure these currents? Essentially, these currents represent fluxes.

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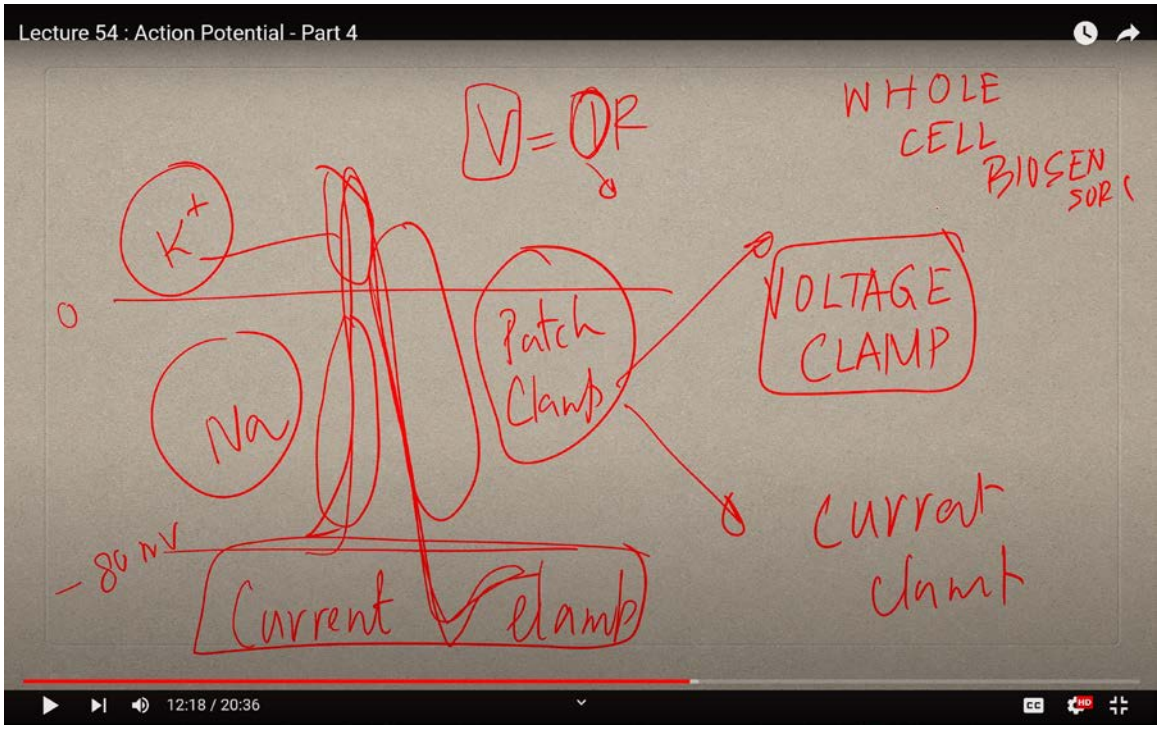

This leads us to two key concepts in the realm of whole-cell biosensors: the voltage clamp and the current clamp. A technique commonly used in this context is called patch clamping, or even intracellular recordings.

So, what is a voltage clamp? The term "clamp" implies that we are holding something steady. In this case, when you clamp the voltage, you can measure the current. This is grounded in Ohm's Law, which states that V = I x R. By fixing the voltage, you can determine the current, or vice versa: by fixing the current, you can measure the voltage.

When you apply a current clamp, you hold the current steady and observe the resulting changes in voltage. Now, let’s explore the voltage clamp method. Imagine we have an electrically active cell, and we place an electrode nearby to measure the current while

maintaining a fixed voltage. This setup is straightforward.

For instance, suppose the cell is initially sitting at -80 millivolts. You would decide on a voltage shift and maintain the voltage constant. So, starting from -80 millivolts, you might apply a pulse to -70 millivolts, then to -60 millivolts. You can adjust this pulse at your discretion, such as shifting it to -75 or -65 millivolts. To simplify this explanation, I'm using increments of 10 millivolts. By doing this, you systematically vary the voltage, fixing it at different points to measure the corresponding currents.

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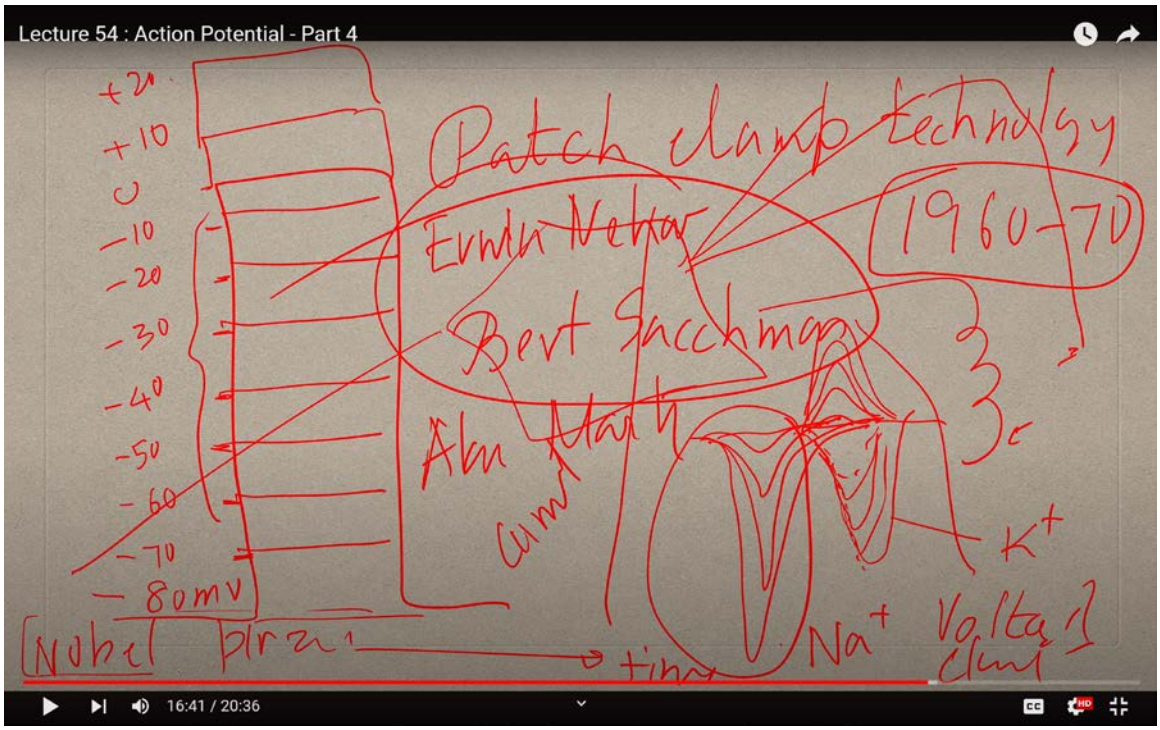

Now, let's dive into the details of the timing involved in this process. As you apply different kinds of pulses, you'll notice a distinct current shifting its value within a specific zone. Eventually, this current will stabilize over time. On the axis you're observing, we are focusing on the current rather than the voltage. Following this initial current, you will observe the emergence of another set of currents that gradually increase. The first set of currents corresponds to the sodium current, while the second set represents the potassium current.

These two currents have different directionalities. I've positioned them as functions of time, but if you wish to represent their directionality differently, you can easily reverse it. In the context of space-time, the sodium current flows from outside the cell to the inside, followed by the potassium current, which flows from the inside to the outside of the cell. This intricate measurement was made possible by a groundbreaking technique known as patch clamp technology, which emerged predominantly in the 1960s and 70s.

This advancement was significantly influenced by the work of scientists such as Erwin Neher, Bert Sakmann, and Alan Marty, among others. Neher and Sakmann were awarded the Nobel Prize for their pioneering efforts in developing patch clamp technology and achieving remarkable voltage-clamp recordings. Prior to this innovation, most techniques relied on current clamps, where voltage changes were measured.

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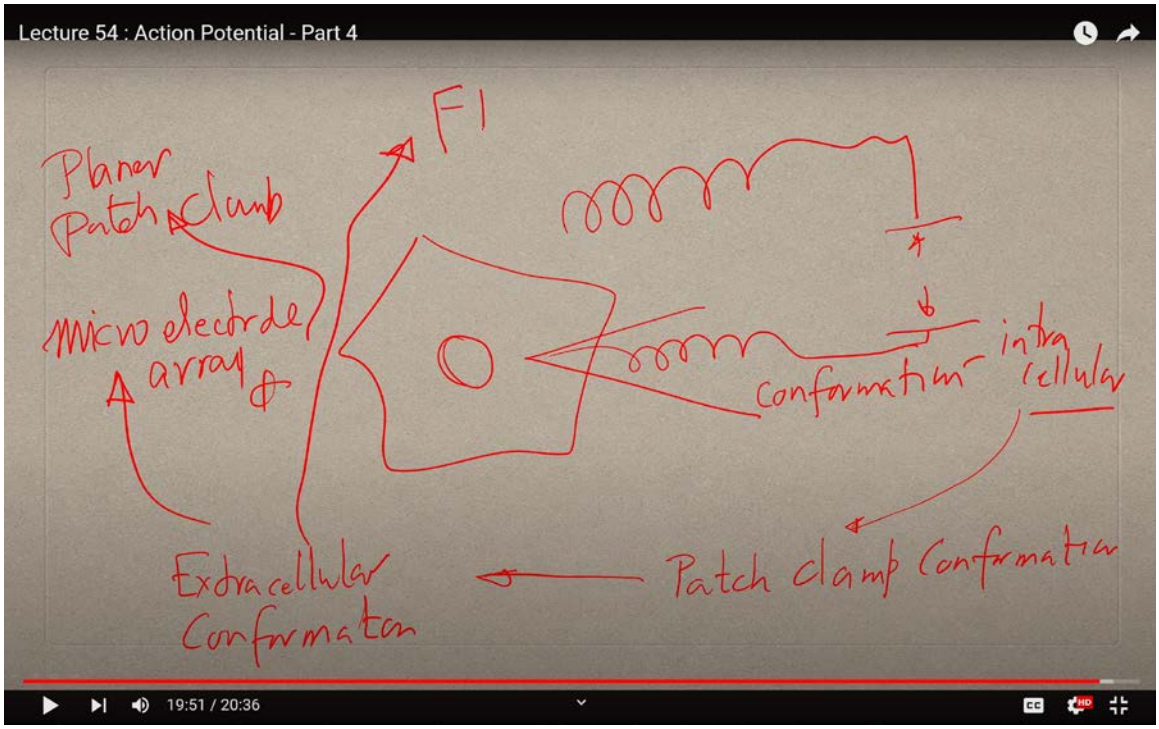

The introduction of patch clamp technology transformed our ability to study excitable cells, marking a critical juncture in the field. This breakthrough dramatically changed the landscape of electrophysiology, as it allowed for precise measurements of sodium and

potassium currents separately, along with their polarity and directionality, something that was previously not feasible. Prior research mainly centered on the current clamp technique, which involved fixing the current and observing voltage changes in millivolts.

The voltage clamp method revolutionized our understanding of voltage-gated ion channels, paving the way for countless developments over the last century. If we look back, we see various configurations of electrodes being utilized. For instance, in the case of an electrically active cell, one configuration is the intracellular setup, where recording electrodes are placed inside the cell, accompanied by a ground electrode for measurements.

In contrast, the second configuration, also categorized as intracellular, involves the electrode being positioned within the cell body, including the nucleus. This represents the first configuration. A refinement of this approach is the patch clamp configuration, which we will discuss in our next class. Additionally, we will explore other configurations used prior to patch clamp technology, specifically the extracellular configuration.

From there, we will advance to topics such as electrode arrays and planar patch clamps. Another fascinating area we will investigate includes field-effect transistors. This is the trajectory our upcoming investigations will take. Thank you for your attention!