**Design for Biosecurity**

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**Lecture 52**

**Action Potential - Part 2**

Let us resume our class where we left off, right at the cusp of discussing the Nernst equation. Today, we will delve into the practical application of this equation and explore why the membrane potential of a cell is measured at approximately -90 millivolts. Understanding this concept will significantly simplify your grasp of action potentials, how they function, and their fundamental prerequisites.

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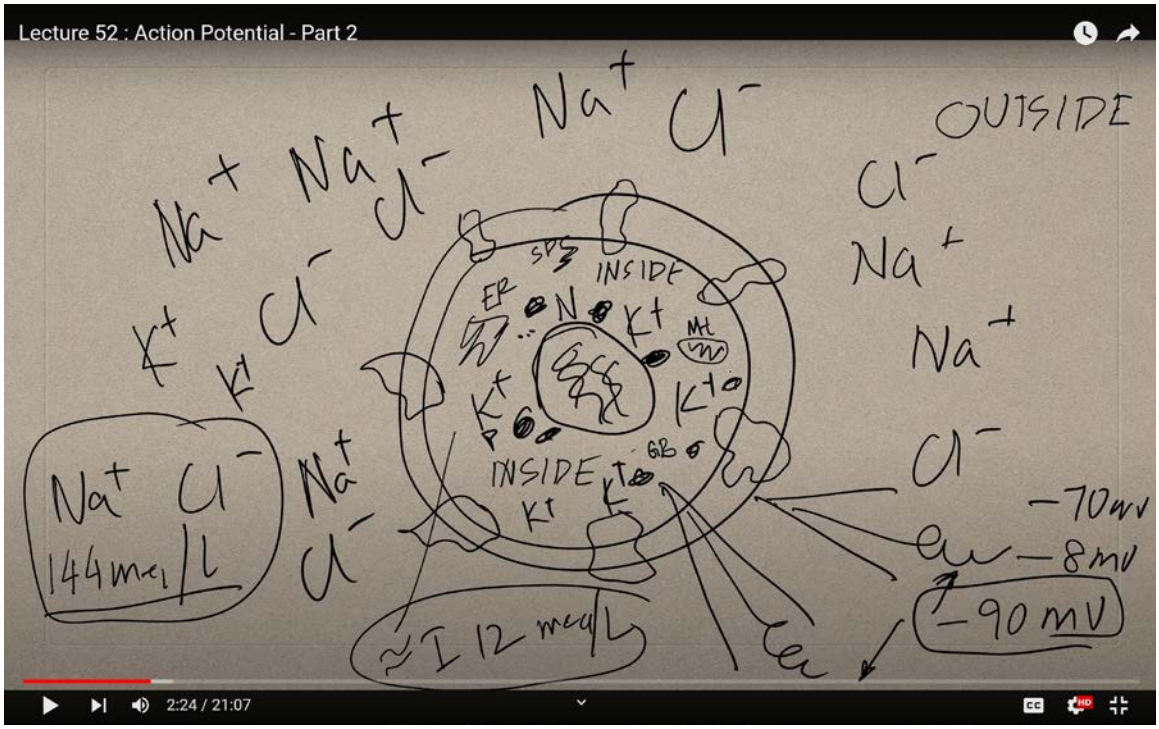


We concluded our previous discussion by noting that cell membrane potentials can range from about -80 millivolts to -70 millivolts, and sometimes even down to -40 millivolts. I am deliberately keeping the details sparse at this stage, but rest assured, we will delve

deeper into the topic soon, and there’s a reason for this approach.

Now, let's focus on the ionic environment outside the cell. Life has evolved such that the concentration of sodium ions ($Na^+$) outside the cell is considerably higher, almost twelve times more than inside. For instance, if the extracellular concentration of sodium and chloride is around 144 milliequivalents per liter, the intracellular concentration is significantly lower, at approximately 12 milliequivalents per liter. This stark difference is crucial to understanding the membrane potential.

As we've discussed, sodium and chloride ions are predominantly found outside the cell, and I'm illustrating this in their ionic dissociated state. On the contrary, potassium ions ($K^+$) are in much higher concentration inside the cell. To clarify, potassium levels outside the cell are relatively low compared to the abundant potassium found within.

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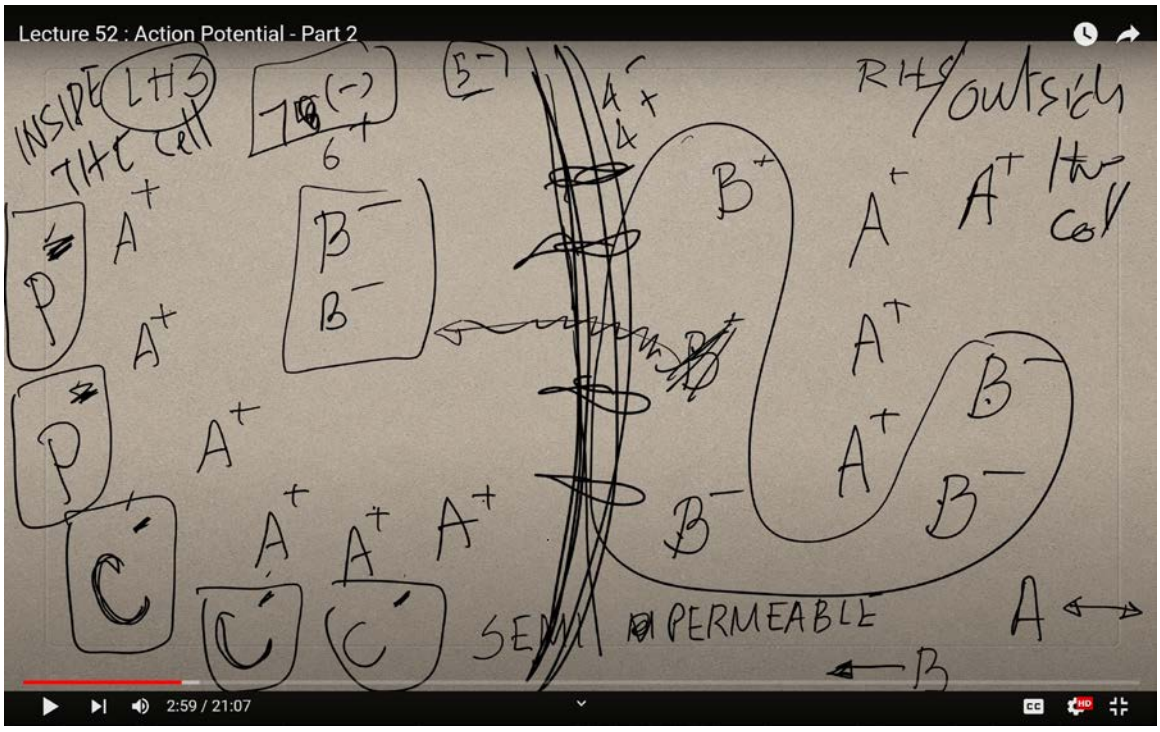


Now, let’s consider an additional factor: within the cell, there are numerous proteins, let's denote them as P’s. Most of these proteins carry a negative charge. Visualize the drawing I provided in the last class, where the left-hand side represents the interior of the cell and

the right-hand side represents the exterior. The membrane in between is semi-permeable, allowing specific ions to flow while restricting others.

Many of the proteins present inside the cell are anchored and cannot migrate across the membrane from the left-hand side to the right-hand side. This anchoring results in a greater accumulation of negative charge within the cell, contributing to the -90 millivolt membrane potential we observe.

To mathematically deduce this potential, we rely on the Nernst equation, which provides a framework for calculating equilibrium potentials. However, acquiring accurate values for sodium and chloride concentrations inside and outside the cell is not straightforward. It requires sophisticated techniques like microiontophoresis, micro-syringes, and microneedles to extract fluid from within the cell and measure these ionic concentrations effectively.

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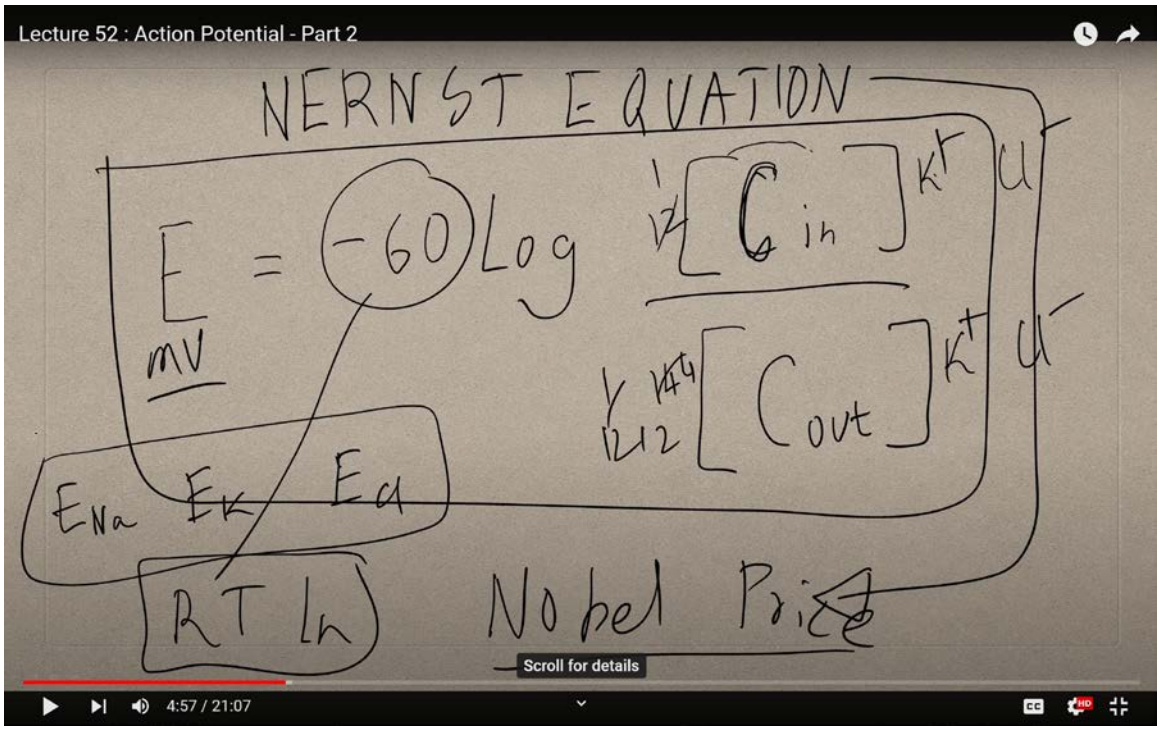


It is indeed a complex process, and you may wonder how we can analyze it. The key lies in the application of the Nernst equation. Now, when we refer to "C," we are discussing

concentration, followed by the logarithmic function, and ultimately, the resulting millivolt potential, right? So, let's say we have a sodium concentration of 12 milliequivalents per liter inside the cell and 144 milliequivalents per liter outside. This gives us a ratio of 12 to 144, or, simplified, 1 to 12.

If you plug these values into the equation, you'll be able to calculate the potential difference specifically for sodium ions. Similarly, you can apply the same method for potassium ions and chloride ions, both inside and outside the cell. As a result, you will derive three distinct values: $E_{Na}$, $E_K$, and $E_{Cl}$. If there are any additional ions present, you can include those as well. By performing this exercise, you will arrive at a particular value, depending on the concentrations you input. This is the remarkable beauty of the Nernst equation!

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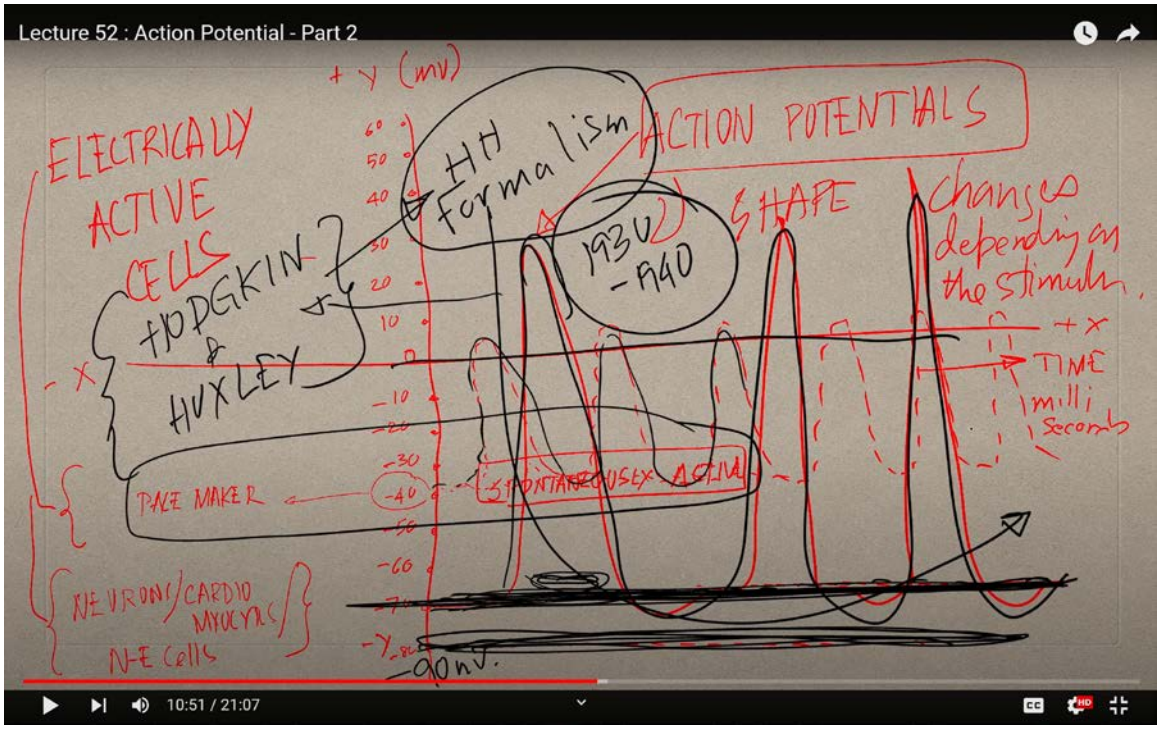


Now, let's extend our understanding to the Goldman, Hodgkin, and Katz (GHK) equation. The GHK equation is essentially an algebraic extension of the Nernst equation. You can express it as the sum of the individual equilibrium potentials: $E_{Na} + E_K + E_{Cl}$. When you add these together using the GHK equation, you arrive at the total membrane potential.

This addition allows us to calculate a combined potential, which can often yield a value around -80 millivolts or -90 millivolts. These fundamental studies have revolutionized our understanding of cellular membrane potentials. For instance, this specific value elucidates why the membrane potential sits at those levels.

Now, you might wonder about the significance of these calculations and how they relate to action potentials. This leads us to a fascinating non-linear phenomenon occurring within the cell. This phenomenon essentially dictates the cell's fate, determining whether it is excitable or non-excitable. So how does this happen?

Based on the principles outlined in the Nernst equation, we have established a resting membrane potential around -80 to -90 millivolts. However, what triggers a spike, causing the membrane potential to rise sharply towards zero, or even overshoot to +10 or +20 millivolts? While this event is transient, it does occur. Understanding this phenomenon was greatly advanced by two British scientists, who meticulously explained the underlying processes.

Prior to their work, a gentleman named Bernstein observed this behavior and likened it to a cell undergoing "load shedding," where it completely goes dark and then suddenly comes back to life. It was Hodgkin and Huxley who provided the in-depth analysis that clarified this intriguing phenomenon.

This is one of the landmark discoveries in the field of bioelectrics, emerging at a time when the world was engrossed in unraveling the structure of DNA, thanks to the efforts of Watson, Crick, and their contemporaries. In that context, prior to this genetic frenzy, Hodgkin and Huxley introduced what is now known as the Hodgkin-Huxley formalism. Remarkably, they accomplished this without any prior knowledge of structural biology or crystallography. At that time, there was no established understanding of membrane proteins, and the very structure of the cell membrane, essentially a lipid bilayer, remained a mystery.

It wasn't until later, in the 1960s, that the theories proposed by Dix and Jolly, along with other contributions, led to the lipid bilayer model we recognize today. The groundbreaking

work of Hodgkin and Huxley, conducted in the 1930s, marked a celebratory period in bioelectronics. Their findings laid the foundation for what we now understand as electrophysiology and biosensors, rooted in these fundamental discoveries of the Hodgkin-Huxley formalism. It's crucial to emphasize that at the time, there was a complete lack of understanding regarding membrane proteins, channels, or pores; their formalism was purely based on the observed movements in sea slugs.

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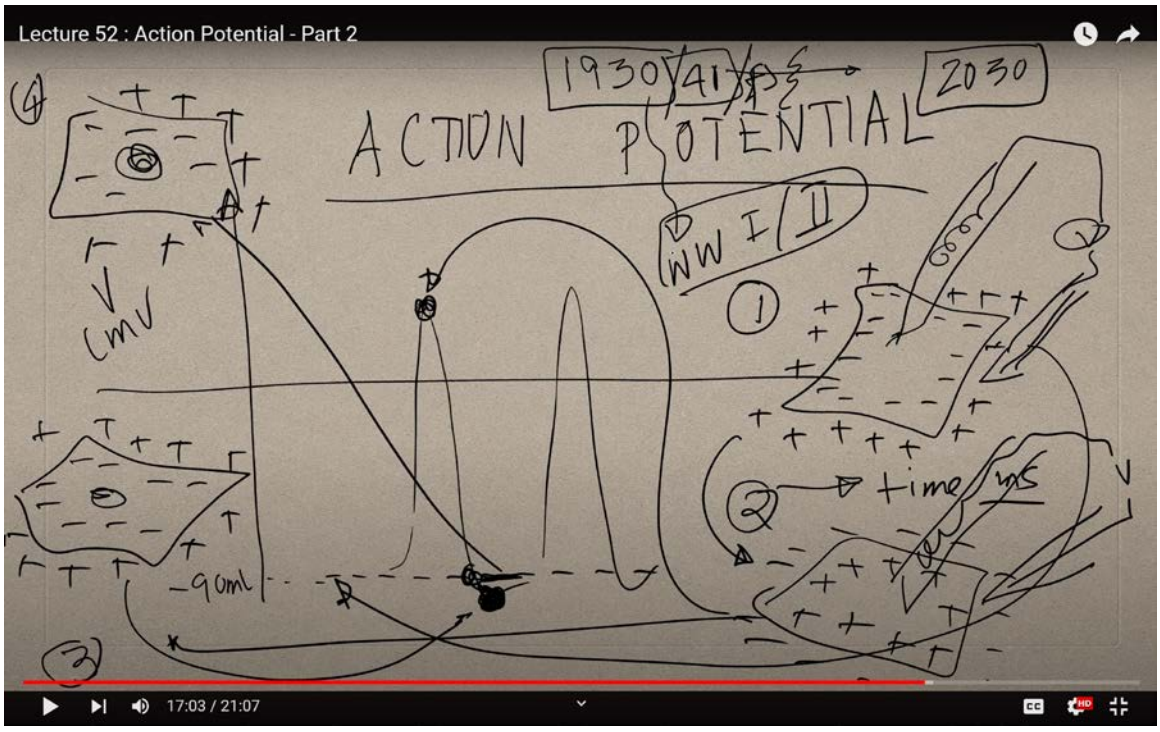


By inserting an electrode into the cell, a technique I illustrated for you, they measured the electrical potential inside versus outside the cell. They manipulated the extracellular fluid and employed the Nernst equation to analyze their results. Utilizing a variety of mathematical tools, they were able to conceptualize the action potential. So, what exactly is action potential?

We previously discussed the Nernst equation, which provided a foundational understanding of why cells generally rest at around -90 millivolts. However, some cells exhibit this fascinating behavior of rapidly "shooting" their voltage, which we can graph

with voltage in millivolts on the vertical axis and time in milliseconds on the horizontal axis.

To comprehend this phenomenon, we must revisit our earlier diagram. This action can only occur if we consider the presence of permanent negative charges represented by the proteins and ions in the cell. These charges cannot simply migrate to the other side of the membrane and leave the cell filled with positive charges. After all, these molecules are substantial and cumbersome; it's hard to believe they could easily travel across the membrane due to their size and mass.

So, how can we explain this rapid change in potential? The key lies in the transient movement of positive charges into the cell. This movement allows for the temporary observation of a positive charge inside the cell alongside a negative charge outside. If we maintain this configuration and examine it closely, we can understand the underlying dynamics. Initially, we might read one potential, and then suddenly, we observe a dramatic shift. This is what allows us to grasp the complexity of action potential.

Initially, we have a scenario where negative charges are positioned in relation to a battery, and then, suddenly, you observe a different situation, a dynamic shift in the electrical landscape. This is precisely where you are measuring at a given point, right? Of course, the electrode is configured to assess the voltage both outside and inside the cell.

Soon after that, we transition through various stages: Stage 1, Stage 2, and now we arrive at Stage 3, where we find ourselves even more negative. In Stage 4, the narrative repeats with positive charges manifesting again. Ultimately, we return to the baseline, observing the baseline once more. So, what truly happened during this sequence of events?

As I mentioned earlier, those large proteins and organelles, which harbor significant negative charges, cannot simply exit the cell. Their movement is crucial, without it, nothing would happen. Now, let's consider the most plausible molecule that could contribute to this dynamic. Given that potassium levels are low outside the cell, the movement of a few potassium ions into the cell wouldn't dramatically alter its internal environment. The real game-changer is sodium, the ion that can flood into the cell and carry a substantial positive

charge with it.

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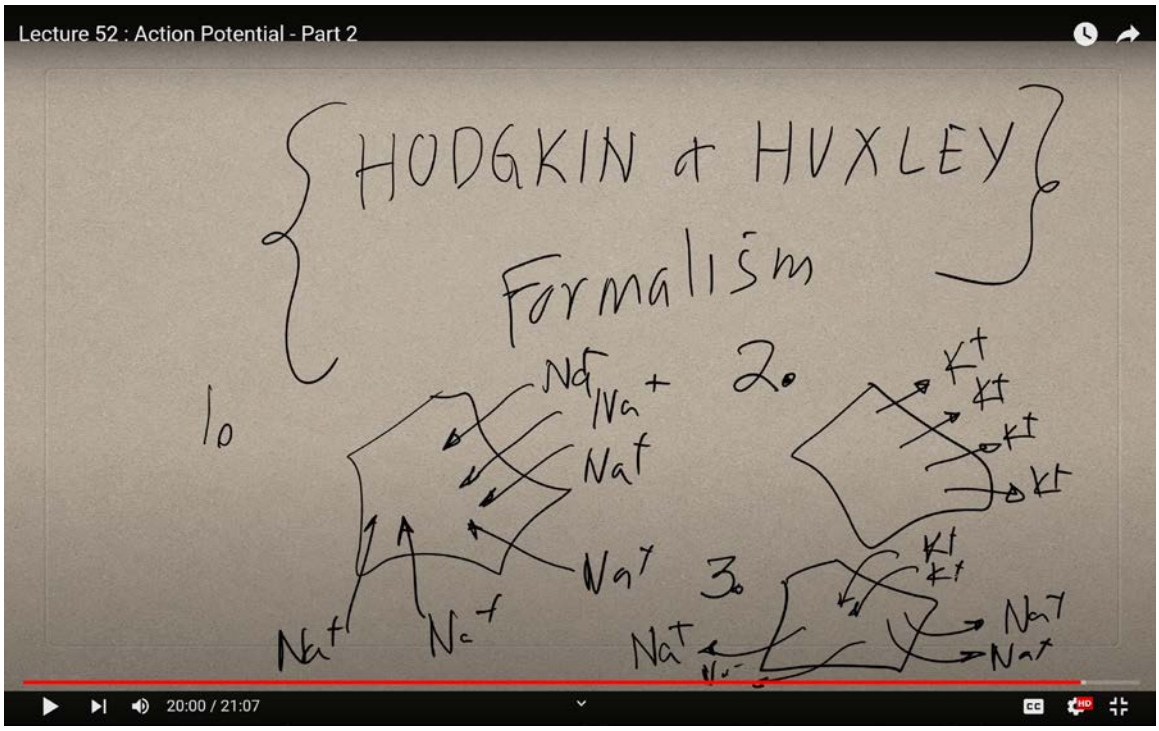


As I noted earlier, if you were to quantify the sodium concentration, you'd find that it is abundant outside the cell. This leads us to a logical conclusion: a significant influx of sodium ions into the cell occurs momentarily. Now, when this influx happens, we must ask whether this is accompanied by a corresponding outflow of potassium ions, or if sodium ions simply enter and then eventually exit.

Let’s remember, this context is rooted in the 1930s. Fast forward almost a century to 2030, and consider the technological advancements we've made since then. However, during the 1930s and 1940s, the world was grappling with the aftermath of two world wars, and the scientists endeavoring to unravel these questions, Hodgkin and Huxley, were, in fact, Royal Air Force pilots. Their remarkable contributions during such turbulent times laid the groundwork for our understanding of cellular dynamics.

Now, how do we justify what is happening within the cell? As I previously mentioned, there is a significantly higher concentration of potassium outside the cell. When potassium

ions move out, the interior of the cell becomes increasingly dominated by sodium. If this continues unchecked, the cell will no longer be able to function or sustain itself; it will effectively desiccate. Do you recall when I asked you to revisit a particular aspect of our previous discussion, where I mentioned a “shelter” that helps the cell survive in a high sodium environment?

If sodium were to remain trapped inside the cell while potassium is allowed to escape in order to maintain balance, we would inevitably be unable to observe a second action potential. The cell would gradually dry out, perhaps capable of a couple of action potentials at most, before it ultimately succumbs to this imbalance.

However, the remarkable thing is that the cell does not simply die off; it continues to generate action potentials over an extended period of time. How is this even feasible? This is where the groundbreaking work of Hodgkin and Huxley comes into play. They made some incredible discoveries regarding this entire process, identifying three key elements.

Step One: To illustrate, a substantial influx of sodium occurs suddenly. I will delve deeper into the reasons behind this influx later on.

Step Two: But that’s not the whole story. There’s also a significant efflux of potassium. Hodgkin and Huxley discovered this mechanism, although at the time, they had no knowledge of the pumps involved in these processes. Sodium is expelled from the cell, while potassium is reintroduced.

In total, there are three critical events taking place that we will discuss in detail in our next class. Understanding these events is essential for grasping how action potentials are generated, which is a cornerstone of excitable cell biology.

Thank you for your attention, and I look forward to exploring this fascinating topic further with you in our next session.