Starting physiology: bioelectrogenesis

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Baptista V. Starting physiology: bioelectrogenesis. Adv Physiol Educ 39: 397–404, 2015; doi:10.1152/advan.00051.2015.—From a Cartesian perspective of rational analysis, the electric potential difference across the cell membrane is one of the fundamental concepts for the study of physiology. Unfortunately, undergraduate students often struggle to understand the genesis of this energy gradient, which makes the teaching activity a hard task for the instructor. The topic of bioelectrogenesis encompasses multidisciplinary concepts, involves several mechanisms, and is a dynamic process, i.e., it never turns off during the lifetime of the cell. Therefore, to improve the transmission and acquisition of knowledge in this field, I present an alternative didactic model. The design of the model assumes that it is possible to build, in a series of sequential steps, an assembly of proteins within the membrane of an isolated cell in a simulated electrophysiology experiment. Initially, no proteins are inserted in the membrane and the cell is at a baseline energy state; the extracellular and intracellular fluids are at thermodynamic equilibrium. Students are guided through a sequence of four steps that add key membrane transport proteins to the model cell. The model is simple at the start and becomes progressively more complex, finally producing transmembrane chemical and electrical gradients. I believe that this didactic approach helps instructors with a more efficient tool for the teaching of the mechanisms of resting membrane potential while helping students avoid common difficulties that may be encountered when learning this topic.

The Didactic Model

As shown in Fig. 1, the didactic model is based on a single isolated cell submersed into an electrolyte solution bath and an appropriate measuring device for \( \Delta V_m \) is used, just as in a real electrophysiology experiment. However, this is a fictitious situation, which assumes that a lipid bilayer sheet, freely floating in an electrolyte solution, has suddenly engulfed a portion of the surrounding fluid and spontaneously formed a cell-like spherical structure with all the relevant intracellular machinery. Under these imaginary conditions, we have to consider that 1) the intracellular fluid (ICF) and extracellular fluid (ECF) have the same ionic composition and 2) the plasma membrane initially consists of a simple phospholipid bilayer, i.e., without proteins. In addition, the didactic model also assumes that it is possible to control membrane proteins, i.e., we can insert or remove them. This is just the starting point for the student’s understanding of bioelectrogenesis mechanisms.

Initial Considerations

Before we start building up the generation of \( \Delta V_m \), it is important to remember some key concepts:

- Electric charges (\( Q \)) of the same sign repel each other, whereas those of opposite signs attract each other with electric force (\( F \)). According to Coulomb’s law (\( F \propto Q_1 \times Q_2 / d^2 \)), these interactions decrease with the square of the distance (\( d \)).
- The electric potential difference (\( \Delta V \)) is related to the spatial separation of electric charges. There is no \( \Delta V \) between two circumscribed areas, \( A \) and \( B \), if both of them have the same net charge, as determined by the number of positive and negative charges in each place. However, if an external force, overcoming the coulomb attraction, translocates one charge from \( A \) to \( B \), \( \Delta V \) is developed between them. Since \( \Delta V \) equals the energy (\( U \)) spent by the translocated charge (\( \Delta V = U/Q \)), the higher the number of separated electric charges, the higher the resulting \( \Delta V \).
- ECF and ICF are electrolyte solutions and therefore electrically conductive. Furthermore, both fluids are primarily electroneutral, i.e., the concentration of cations = the concentration of anions. \( \text{Na}^+ \) and \( \text{Cl}^- \) are the main extracellular ions, whereas \( \text{K}^+ \), \( \text{P} \), and anionic proteins are the main intracellular charge carriers. Here, for simplicity, the anions are not represented in the figures, but we have to keep in mind that they are present in equivalent charge to those of the cations.
- The thermodynamic variables (pH, temperature, osmolarity, etc.) of the ECF are kept constant by homeostatic mechanisms. For our purpose, it is important to note that 1) \( \text{Na}^+ \) concentration is kept high (~142 mM) relative to \( \text{K}^+ \) concentration (~4 mM), as shown in Fig. 1, where the relative
size of the letters in the ion concentration indicates the magnitude of the concentration, and 2) the ECF is constantly supplied with glucose, O₂, and amino acids.

• According to kinetic theory, ions and molecules in the ECF, driven by thermal agitation \(3/2 k T\), where \(k\) is a constant and \(T\) is the temperature (in Kelvin), randomly crash against the outer surface of the membrane. Hence, it is easy to figure out that at a given \(T\), the higher the concentration of a particular ionic species \(i\) \(([i])\), the greater the frequency of collisions of this species with the membrane. The relationship among \(T\), \([i]\), and the probability of such collisions can be referred to as the chemical potential of \(i\) \((\mu_i)\) and is expressed as \(\mu_i = R / T \ln ([i])\), where \(R\) is the gas constant \((8.31 \text{J/K-mol})\). Note that if \(T\) is kept constant, \(\mu_i\) depends only on \([i]\); the higher \([i]\), the higher the probability of collisions. In this sense, we can therefore think that \(\mu_i\) of the ECF is the tendency of \(i\) to cross the membrane from the ECF to the ICF driven by thermal agitation.

• However, since the core of the cell membrane is hydrophobic, this structure is an insulating material. This means that when an ion hits the membrane, it does not penetrate the membrane.

• Due to 1) the insulating properties and well-defined planar geometry of the membrane, 2) the thinness of the membrane, and 3) the electrical conductivity of the biological fluids, the ECF-membrane-ICF triad is a capacitor. This means that if the electric potential energy of attraction overcomes the kinetic energy, opposite unpaired electric charges in both fluids will attract each other across the membrane, generating a transmembrane electric field.

• Because the electric force is conservative, an electric potential energy \((U)\) is stored in the electric field of the membrane, which is given by \(U = z F \Delta V\) (where \(z\) is the valence of the charge and \(F = 9.6 \times 10^4 \text{C/mol}\)).

Considering the above, we can infer that for a nonzero \(\Delta V_m\) to exist the following requirements are necessary: 1) the presence of mobile and opposite charges, 2) a force to separate them, 3) a conducting pathway so that they flow separately from each other, and 4) a device like a capacitor to keep them apart. In a system made up by a living cell and its surrounding fluid, these four elements are very well organized, so a \(\Delta V_m\) ranging from \(-5\) to \(-100\) mV, depending on the type of cell, is established. Here, we will interconnect these requirements to elevate \(\Delta V_m\) from 0 mV (Fig. 1) to \(-100\) mV (Fig. 5), through a series of four basic steps, as follows.

**Bioelectrogenesis Steps**

**Step 1: generation of Na⁺ and K⁺ concentration gradients.**

Let’s start from the “reset” or “unloaded” cell shown in Fig. 1, where the membrane has no proteins and ICF = ECF (note, also, that \(\Delta V_m = 0\) mV). We can say that the system is at the baseline energy state. In such a state, both fluids are at thermodynamic equilibrium so for any net change in the fluids’ composition, it is necessary to supply some work to the system.

Of course, the cell membrane is not exclusively composed of lipid molecules; there is a vast array of different proteins embedded in the membrane accounting for a number of different functions. Na⁺-K⁺-ATPase (or pump), first reported by J. C. Skou (21), is a transmembrane protein present in almost all animal cells. This protein is an active transport system that uses metabolic energy to move Na⁺ out and K⁺ in vectorially across the membrane against their concentration gradients. The stoichiometry of the pump is 3Na⁺:2K⁺:1ATP, and its strongest activator is a high intracellular Na⁺ concentration \(([Na^+]_{in})\), so it will be quite active under the condition shown in Fig. 1.

Regarding our didactic model, certain membrane proteins can be inserted to serve specific functional roles. In this way, the major factors that underlie the genesis of \(\Delta V_m\) can be clearly identified. Therefore, to start the process of bioelectrogenesis, we can now insert the Na⁺-K⁺-ATP pump into the membrane (the small circle in Fig. 2A, which represents the combined effect of thousands of pumps scattered over the membrane). The initially high \([Na^+]_{in}\) induces a high turnover rate of the pump. Progressively, Na⁺ is extruded and K⁺ is taken up, resulting in a decrease in \([Na^+]_{in}\) and an increase in intracellular K⁺ concentration \(([K^+]_{in})\), as shown in Fig. 2A, where the size of the letters is correlated with concentration. As pointed out, the activity of the pump is a function of \([Na^+]_{in}\), such that as \([Na^+]_{in}\) decreases, the turnover rate of the pump slows down. But, note also in Fig. 2A that a small \(\Delta V_m\) (equal to \(-1\) mV) is forming. This happens because the number of ions transported by the pump is not equal in each direction (3Na⁺ out:2K⁺ in); the uneven translocation of charges generates a net outward Na⁺ current across the membrane. One net positive charge flows outwards per pump cycle, and, consequently, a negative unpaired charge is left trapped inside the cell by the lipid membrane. Since the system operates as a capacitor, the opposite unpaired charges do not zigzag randomly through the fluids; instead, they attract each other on either side of the membrane, forming a thin cloud of negative charges scattered along the inner surface of the membrane and a positively charged cloud on the outer surface. The end result of the pump’s work, in addition to the generation of Na⁺ and K⁺ gradients, is a slight separation of charges across the membrane that gives rise to a small \(\Delta V_m\). In a typical mammalian neuron, the pump activity maintains an \(~14\)-fold gradient for Na⁺ and \(~35\)-fold gradient for K⁺. For the model cell, the working pump establishes \([K^+]_{in} = 140\ \text{mM}\), \([Na^+]_{in} = 10\ \text{mM}\) and \(\Delta V_m = \sim 2.5\) mV (Fig. 2B). Note that,
in the ICF the same amount of unpaired negative charges. Due to electrical work of the pump carries positive charges to the ECF, leaving behind the vertical arrows and by the size of the letters for the \[ion\]. The unpaired 

\[35, \text{ extracellular } [\text{Na}^+]/[\text{K}^+]\]

is infinitely larger than the intracellular compartment; thus, Na\(^+\) (the white ring in Fig. 3A). At the same time, we will remove the Na\(^+-\)K\(^+\) pump so we can analyze the role of K\(^+\) channels separately. We assume that these channels, referred as resting K\(^+\) channels, are always open, so K\(^+\) can diffuse freely through them at all times from the ICF to the ECF and vice versa. The K\(^+\) channels that we have inserted represent, of course, the combination of a large number of them scattered over the membrane. In this new configuration, the chemical potential of intracellular K\(^+\) ([\([\mu_{K^+}]_{\text{in}}\)]) drives K\(^+\) to the extracellular space through K\(^+\) channels with a “force” equal to \(R \times T \times \ln{[\text{K}^+]_{\text{in}}}\). Similarly, the chemical potential of extracellular K\(^+\) ([\([\mu_{K^+}]_{\text{out}}\)] causes K\(^+\) influx (\(R \times T \times \ln{[\text{K}^+]_{\text{out}}})\). Because \([\text{K}^+]_{\text{in}} > [\text{K}^+]_{\text{out}}\) it follows that \(([\mu_{K^+}]_{\text{in}} > \)

\(\text{Step 2: } K^+ \text{ conductance.}\) Unlike a pure lipid bilayer, the typical cell membrane has ion conductance pathways, so that it acts as a leaky capacitor. Ion channels, a type of transmembrane protein, consist of hydrophilic microdomains that allow ions to diffuse across the membrane. Furthermore, ion channels are selective for different ionic species.

Applying this to the didactic model and to mimic a real cell, which has a membrane highly permeable to K\(^+\), let us insert selective K\(^+\) channels within the membrane of the model cell (the white ring in Fig. 3A). At the same time, we will remove the Na\(^+-\)K\(^+\) pump so we can analyze the role of K\(^+\) channels in the process, neither extracellular Na\(^+\) concentration ([Na\(^+\)]\(_{\text{out}}\)) nor extracellular K\(^+\) concentration ([K\(^+\)]\(_{\text{out}}\)) of the bulk solution are significantly altered; any tendency in this direction is canceled by the homeostatic systems of the body. (In the model, we can assume the extracellular space to be infinitely larger than the intracellular compartment; thus, Na\(^+\) and K\(^+\) concentrations of the surrounding fluid are not altered by the pump.) Furthermore, we have to consider that only a small imbalance in ion electroneutrality close to the membrane surface is required to generate physiological values of \(\Delta V_m\) due to the function of the membrane as an excellent capacitive device that allows charge separation.

**Fig. 2. Bioelectrogenesis step 1:** generation of Na\(^+\) and K\(^+\) chemical gradients. \(A\): the insertion of the Na\(^+-\)K\(^+\) pump (small circle) establishes the active transport of Na\(^+\) and K\(^+\), which progressively increases intracellular [K\(^+\)] ([K\(^+\)]\(_{\text{in}}\)) and decreases intracellular [Na\(^+\)] ([Na\(^+\)]\(_{\text{in}}\), as indicated by the vertical arrows and by the size of the letters for the [ion]. The unpaired electrical work of the pump carries positive charges to the ECF, leaving behind the same amount of unpaired negative charges. Due to electrical attraction, the unpaired opposite charges accumulate close to the surfaces of the membrane, giving rise to a small electrical potential difference across the cell membrane (\(\Delta V_m\)), as registered by the voltmeter. \(B\): as the pump’s work continues, the ICF becomes different to the ECF; at the pump’s maximal transport capacity, the [ion] ratios are [K\(^+\)]\(_{\text{out}}/[\text{extracellular } [\text{K}^+] \rightleftarrows [\text{K}^+]_{\text{in}} = 35, \text{ extracellular } [\text{Na}^+] ([\text{Na}^+]_{\text{out}})]/[\text{Na}^+]_{\text{in}} = 14, \text{ and } \Delta V_m \approx -2.5 \text{ mV}.

**Fig. 3. Bioelectrogenesis step 2:** the role of resting K\(^+\) conductance. \(A\): upon the insertion of a K\(^+\) channel (white ring), the K\(^+\) chemical potential difference (\(\Delta \mu_{K^+} \approx 9.189 \text{ J/mol}\)) drives K\(^+\) influx (solid arrow) and the electrical potential energy (\(U = 240 \text{ J/mol}\)) drives an inward flux (dotted arrow). As \(\Delta \mu_{K^+} > U\), the resultant K\(^+\) movement is outward, as indicated by the length of the arrows. \(B\): the ongoing resultant outward K\(^+\) flux carries positive charges to the ECF. This is a process of charge separation that increases \(\Delta V_m\), which, in turn, increases K\(^+\) influx driven by \(U\), as indicated by the increasing length of the dotted arrow. \(C\): as the net K\(^+\) influx progresses, a steadily increasing number of electric charges is separated, increasing \(\Delta V_m\) up to \(U = \Delta \mu_{K^+}\). At this point, the influx of positive electric charges is equal to their efflux and \(\Delta V_m\) does not change; the cell has reached stationary equilibrium.

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(\(\mu_{K^+}\))\text{\_out}, and therefore, the net K\(^+\) movement is outwardly directed. Quantitatively, the net K\(^+\) movement across the membrane is a function of the difference of \(\mu_{K^+}\) between the ICF and ECF, which is \((\mu_{K^+})\text{\_in} - (\mu_{K^+})\text{\_out} = \Delta\mu_{K^+} = R \times T \times \ln([K^+]\text{\_in}/[K^+]\text{\_out}).\) Then, the resultant K\(^+\) efflux driven by \(\Delta\mu_{K^+}\) is represented here by a single solid arrow directed outward, as shown in Fig. 3.

Note that the resultant efflux of K\(^+\) is a spontaneous phenomenon that drives the system toward \(\Delta\mu_{K^+} = 0\), i.e., \([K^+]\text{\_in} = [K^+]\text{\_out} \). However, this tendency is counteracted by \(U\) stored in the electric field of the membrane, which also drives K\(^+\) through the selective channels (recall that \(U\) is directly proportional to \(\Delta V_m \approx -2.5\) mV generated by the Na\(^+\)-K\(^+\) pump). Because the inside of the cell is negative relative to the outside, K\(^+\) driven by \(U\) migrates unidirectionally, making an inward K\(^+\) current (dotted arrow in Fig. 3A), which tends to counteract the outwardly directed K\(^+\) current generated by \(\Delta\mu_{K^+}\).

Therefore, there are two types of potential energy able to move ions across the membrane: chemical and electrical. [The algebraic sum of these two parameters is called the electrochemical potential difference of an ionic species \((\Delta\mu_i)\)] considering \([K^+]\text{\_in}/[K^+]\text{\_out} = 35, \Delta V_m \approx -2.5\) mV, and \(T = 37^\circ C = 310\) K, \(\Delta\mu_{K^+}\) stored in the K\(^+\) gradient is \(\approx 9.158.9\) J/mol, whereas \(U\) stored in the electric field of the membrane is \(-240\) J/mol. The opposite signs indicate the opposite direction of the gradients, and, as expected, the magnitude of the gradients is directly proportional to the intensity of the K\(^+\) movements, as indicated by the directions and lengths of the arrows in Fig. 3A. Therefore, the amplitude of the outward K\(^+\) current is larger than that of the inward current. In these conditions, the inward current driven by \(U\) only partially counteracts the outward current driven by \(\Delta\mu_{K^+}\), so the rate of transference of positive charges out of the cell exceeds the inward translocation. This means that, since the membrane permeability is selective to K\(^+\), the net outward K\(^+\) current leaves behind in the ICF an equal number of negative unpaired charges. Hence, because of the capacitive properties of the membrane, the unpaired negative and positive charges are pulled toward the membrane surfaces by electrostatic attraction. In other words, the resultant outward K\(^+\) current progressively increases the density of unpaired charges on the membrane surfaces, leading to a proportional enhancement of \(\Delta V_m\) beyond of the \(-2.5\) mV generated by the Na\(^+\)-K\(^+\) pump.

However, note that the net outward K\(^+\) flux is a self-limiting process. The gradual increase in \(\Delta V_m\) (which reaches \(-50\) mV in Fig. 3B) comprises an increase in the electric driving force that pulls K\(^+\) back into the cell. The result is a gradual increase in the inward K\(^+\) current as \(\Delta V_m\) increases, as shown by the increased length of the dotted arrow in Fig. 3B. Then, it is clear that, as the hyperpolarization progresses, the increasing inward K\(^+\) current will eventually cancel out the stable outward current (Fig. 3C); at that moment, \(U\) equals \(\Delta\mu_{K^+}\). At this point, the cell is at stationary equilibrium, i.e., there is a K\(^+\) current in both directions across the membrane but, because they are of identical amplitude, there is no net current and, therefore, \(\Delta V_m\) does not change. This dynamic equilibrium can be quantified by considering that the two gradients cancel each other out, so \(\Delta\mu_{K^+} + U = 0\). In extended form, and solving for \(\Delta V_m\), we write \(\Delta V_m = -(R \times T \times \ln([K^+]\text{\_in}/[K^+]\text{\_out}) \times \ln([K^+]\text{\_in}/[K^+]\text{\_out}).\) This is the Nernst equation. It predicts the amplitude of the electric potential that must exist across the membrane to exactly counteract the K\(^+\) chemical potential.

By applying the Nernst equation to our experimental conditions,

\[
\Delta V_m = -\frac{8.31 \times 310}{1 \times 9.6 \times 10^5} \times \ln\left(\frac{[140 \times 10^{-3}]}{[4 \times 10^{-3}]^2}\right) \approx -95.4\ mV
\]

At this value, \(\Delta V_m \approx -95.4\) mV (Fig. 3C), \(U \approx 9,158.9\) J/mol) exactly equals \(\Delta\mu_{K^+}\), keeping the cell at a stationary state. That is to say, if \(T\) and \([K^+]\text{\_in}/[K^+]\text{\_out}\) are kept constant, \(\Delta V_m\) is no longer time dependent.

The \(\Delta V_m\) predicted by Nernst equation is referred to as the equilibrium potential of a given ionic species \(i\) (\(E_i\)). We can apply the Nernst equation to any ionic species that is chemically unbalanced across the membrane. If the membrane is permeable to only one ionic species, the membrane potential will be equal to the equilibrium potential of that species. In our experiment, \(\Delta V_m = E_K \approx -95.4\) mV. Note that \(E_i\) can be considered the electrical representation of \(\Delta\mu_i\).

It is important to note that the amount of K\(^+\) that flows out of the cell required to build \(\Delta V_m \approx -95.4\) mV is minimal compared with the total K\(^+\) in the ICF. The low dielectric constant of the membrane allows the development of large \(\Delta V_m\) for a given density of separated opposite charges. This means that \([K^+]\text{\_in}\) is not significantly altered during the \(\Delta V_m\) generation (then, in the figures, the solid arrows that indicate flow generated by \(\Delta\mu\) never change in length).

Step 3: Na\(^+\) Conductance. Most cells have membranes not exclusively permeable to K\(^+\); instead, they present multiple conductances, which can also contribute to the generation of \(\Delta V_m\). Resting Na\(^+\) channels, the second most important contributor to \(\Delta V_m\), are highly selective for Na\(^+\) and are always open, allowing Na\(^+\) to move across the membrane in both directions.

To understand the role of Na\(^+\) movements, we will switch on Na\(^+\) conductance (\(g_{Na}\)) by inserting a Na\(^+\) channel into the membrane (the rectangle in Fig. 4). Using the same reasoning for K\(^+\) flux, we realize that both the difference in chemical potential for Na\(^+\) (\(\Delta\mu_{Na^+}\)) and \(U\) drive Na\(^+\) in the same direction, from the ECF to the ICF, as shown by the arrows inside the Na\(^+\) channel (Fig. 4A).

Here, a quantitative comparison is required between Na\(^+\) and K\(^+\) movements. Since these ions are both monovalent, \(U\) drives them across the membrane with equal energy. However, the dotted arrow in Fig. 4A, indicating K\(^+\) influx driven by \(U\), is longer than the dotted arrow corresponding to Na\(^+\) influx, also driven by \(U\). The reason is simple: Na\(^+\) conductance \((g_{Na})\) is much higher than \(g_{Na}\). In other words, the resistance of the membrane to Na\(^+\) flux is higher than that for K\(^+\), with the reason being that the K\(^+\) channels are more conductive and more numerous than Na\(^+\) channels. For ionic fluxes driven by \(\mu\) the same reasoning applies: although in our experimental conditions \(\Delta\mu_{Na^+} \approx 6,835\) J/mol is close to \(\Delta\mu_{K^+} \approx 9,158.9\) J/mol, K\(^+\) flux is much larger than Na\(^+\) flux due to \(g_{Na} >> g_{K^+}\).

Considering the above, it is easy to see that upon the insertion of the Na\(^+\) channel, the steady-state condition shown in Fig. 3C is broken. Now, as shown in Fig. 4A, there is a net inward current driven by \(\Delta\mu_{Na}\), which changes \(\Delta V_m\) accordingly. Recall that because Na\(^+\) channels are selective, the net influx of positive charges leaves behind in the ECF the same amount of unpaired negative charges. Then, the positive charges flowing into the cell...
is, in fact, a net Na

Nernst equation in our experimental conditions). The difference (\(\Delta V_m\)) indicates the driving force of the respective ionic current (\(i_{Na}^+\)).

Fig. 4. Bioelectrogenesis step 3: the role of resting Na\(^+\) conductance. A: upon the insertion of the Na\(^+\) channel (rectangle), the Na\(^+\) chemical potential difference (\(\Delta \mu_{Na^+}\)) and \(U\) drive Na\(^+\) influx (both arrows inside the Na\(^+\) channel point inward). B: the net positive electric charges carried by the inward Na\(^+\) movement discharge the membrane capacitor, which decreases \(\Delta V_m\) and, consequently, the electrical driving force, i.e., \(U\) (note the decrease in the length of the dotted arrows). The discharging of the membrane continues until the influx of positive charges equals once again their efflux (the total lengths of all inward arrows would equal the length of the outward arrow). At this point, \(\Delta V_m = -87.4\) mV, the cell is at a “pseudo”-stationary equilibrium: there is, in fact, a net Na\(^+\) influx and a net K\(^+\) efflux, which tend to decrease the chemical gradients.

cancel out the same amount of negative charges on the membrane capacitance. At the same time, the unpaired negative charges left in the ECF cancel out the same amount of positive charges on the external surface of the membrane. This process progressively discharges the membrane capacitor, decreasing \(\Delta V_m\) with a tendency toward \(E_{Na^+} = +71.2\) mV (as predicted by the Nernst equation in our experimental conditions).

However, this tendency toward \(E_{Na^+}\) is quickly counteracted by a steadily increasing K\(^+\) efflux, which arises when \(\Delta V_m\) deviates from \(E_{K^+}\). Note that as the membrane depolarizes (due to Na\(^+\) influx), \(U\) decreases proportionally, so inward K\(^+\) and Na\(^+\) currents driven by \(U\) also decrease, as shown by the smaller length of the dotted arrows in Fig. 4B. As a consequence, the inward Na\(^+\) current progressively decreases, whereas the outward K\(^+\) current increases. Then, we can infer that these opposing fluxes are driving the system to a \(\Delta V_m\) where once again the inward current (\(\mu\)) will equal the outward current (\(\nu\)), i.e., \(-i_{Na^+}(\Delta \mu_{Na^+}) - i_{Na^+}(U) = +i_{K^+}(\Delta \mu_{K^+})\) (within the parentheses is indicated the driving force of the respective ionic current \(i_{ion}\)).

In other words, the net inward Na\(^+\) current equals the net outward K\(^+\) current: \(-i_{Na^+}(\Delta \mu_{Na^+}) = +i_{K^+}(\Delta \mu_{K^+})\). Exactly at this point the cell is at stationary equilibrium, i.e., there is no net current, \(i_{K^+}(\Delta \mu_{K^+}) + i_{Na^+}(\Delta \mu_{Na^+}) = 0\), and, therefore, \(\Delta V_m\) does not change. This dynamic equilibrium can be quantified by applying Ohm’s law: \(i = g \times \Delta V\). However, we have to consider that \(\Delta V_m\) is not the only driving force; \(\Delta \mu\) also drives ions across the membrane. As pointed out and following the Nernst equation, the \(\Delta \mu\) of a given ionic species may be considered, in electric terms, as the \(E\) of that species. Then, taking into account that the ionic flux driven by \(\Delta \mu\) (i.e., \(E\)) always generates a \(\Delta V_m\) driving an opposite flux, the total driving force across the membrane is given by the algebraic difference of these two sources as \(\Delta V_m - E\).

In other terms, if at the equilibrium potential there is no current, it means that \(\Delta V_m = E\), and, consequently, the driving force for an ion is measured by \(\Delta V_m - E\). In accordance with Ohm’s law, the coefficient that relates the driving force to the ionic current is ionic conductance, so that at stationary equilibrium \(g_{K^+} \times (\Delta V_m - E_{K^+}) + g_{Na^+} \times (\Delta V_m - E_{Na^+}) = 0\). Solving for \(\Delta V_m\), we write \(\Delta V_m = (g_{K^+} \times E_{K^+} + g_{Na^+} \times E_{Na^+})/(g_{K^+} + g_{Na^+})\). This is a linearized version of the Goldman-Hodgkin-Katz (GHK) equation, first derived in its standard form by Hodgkin and Katz (10).

This equation predicts the amplitude of the electric potential that must exist across the membrane to exactly counteract the K\(^+\) and Na\(^+\) chemical potentials (here expressed in electric terms as \(E_{K^+}\) and \(E_{Na^+}\), respectively) and that \(\Delta V_m\) is weighted by the relative conductance to each ion. In other words, \(\Delta V_m\) is closer to the equilibrium potential of a given ionic species the greater its conductance compared with other membrane conductances.

Considering that in a typical neuronal cell, under steady-state conditions, \(g_{K^+} \times g_{Na^+}\) is \(\approx 1.05\) (or \(g_{K^+} = 20 \times g_{Na^+}\)), we can rewrite the GHK equation as follows: \(\Delta V_m = (20 \times E_{K^+} + E_{Na^+})/21\). By applying this to the model cell (Fig. 4B), \(\Delta V_m = (20 \times (-95.4 \times 10^-3)) + 71.2 \times 10^-3)/21 \approx -87.4\) mV.

Exactly at this voltage value, the net positive outward current (driven by \(\Delta \mu_{K^+}\)) equals the net positive inward current (driven by \(\Delta \mu_{Na^+}\)) and \(U\) and, therefore, \(\Delta V_m\) does not change. This would then be the resting membrane potential of the model cell. However, the cell in Fig. 4B is at risk since it is drifting away from dynamic equilibrium. Note that although the outward current is equal to the inward current at \(\Delta V_m = -87.4\) mV, there is, in fact, a net efflux of K\(^+\) and a net influx of Na\(^+\). Eventually, these spontaneous leakages tend to vanish as \(\Delta \mu_{K^+}\) and \(\Delta \mu_{Na^+}\) decrease and disappear, leading the cell to the baseline energy state, which means cell death.

**Step 4: Active transport.** To counteract Na\(^+\) and K\(^+\) spontaneous flows, the cell relies on active transport: the Na\(^+\)-K\(^+\) pump. Then, we need to reinsert the pump into the membrane of the model cell (Fig. 5A). In this configuration, while \(\Delta \mu_{Na^+}\) drives a net inward Na\(^+\) current and \(\Delta \mu_{K^+}\) drives a net outward K\(^+\) current, the pump generates opposing fluxes, i.e., outward Na\(^+\) and inward K\(^+\) currents. We can consider that the spontaneous Na\(^+\) influx, which tends to increase [Na\(^+\)]\text{in}, constantly activates the pump turnover rate. In addition, as the pump activity is electrically unpaired (3Na\(^+\)->2K\(^+\)), there is a resultant positive outward current carried by Na\(^+\) that redistributes the charges on membrane capacitance toward hyperpolarization. This displaces the voltage to a new value (\(\Delta V_m \approx -90.0\) mV; Fig. 5B), where active and spontaneous Na\(^+\) and K\(^+\) currents cancel each other such that there is neither a net Na\(^+\) current nor a K\(^+\) current. Note that before the pump starts working (Fig. 4B), the spontaneous currents are balanced, i.e.,
The role of Na\(^+\)-K\(^+\) pump conductance. A: upon its reinsertion, the pump establishes a net outward positive current (carried by Na\(^+\); recall that 3Na\(^+\) out:2K\(^+\) in). B: the membrane is hyperpolarized (\(\Delta V_m = -90.0\) mV) according to the resultant efflux of positive charges. In addition to this small contribution to \(\Delta V_m\), the pump generates (Fig. 2) and maintains chemical gradients: the Na\(^+\) efflux driven by the pump counteracts the Na\(^+\) influx driven by \(\Delta\mu_{Na}\); the K\(^+\) influx driven by the pump counteracts the K\(^+\) efflux driven by \(\Delta\mu_k\), keeping the cell out of thermodynamic equilibrium but at stationary equilibrium.

\[ i_{\text{K}}^* = -i_{\text{Na}}. \]

However, to equilibrate pump currents, the spontaneous Na\(^+\) flux must be, at least some point, 50% higher than the spontaneous K\(^+\) flux. This readjustment is achieved by the hyperpolarization created by the pump that (1) increases \(i_{\text{K}}(U)\), which, in turn, decreases \(\Delta\mu_{\text{K}}\), and (2) increases \(i_{\text{Na}}\), which, in turn, increases \(\Delta\mu_{\text{Na}}\). The result, as shown by the longer lengths of the dotted arrows in Fig. 5B, is a slight decrease in the outward K\(^+\) leakage currents and an increase in the inward Na\(^+\) leakage currents, reaching the ratio of 3/2 \(i_{\text{K}}(-\Delta\mu_{\text{K}}) = -i_{\text{Na}}(\Delta\mu_{\text{Na}})\). This ratio exactly matches the stoichiometry of the pump (3Na\(^+\):2K\(^+\)), bringing the cell back to stationary equilibrium. This dynamic balance can be quantified by considering the ratio of 3/2 \(i_{\text{K}}(-\Delta\mu_{\text{K}}) = -i_{\text{Na}}(\Delta\mu_{\text{Na}})\). In extended form, we have 3/2 \[ \left[ \frac{\Delta V_m}{E_{\text{Na}}} \right] = -\frac{\Delta\mu_{\text{Na}}}{\Delta\mu_{\text{K}}}. \]

By solving for \(\Delta V_m\) and assuming \(\Delta\mu_{\text{Na}} = 20 \times \Delta\mu_{\text{K}}\), we have \(\Delta V_m = (30 \times E_{\text{K}} + E_{\text{Na}})/31\). This equation quantitatively predicts the \(\Delta V_m\) for membranes permeable only to Na\(^+\) and K\(^+\). By calculating for the model cell, \(\Delta V_m = 30 \times (-95.4 \times 10^{-3}) + 71.2 \times 10^{-3})/31 \approx -90.0\) mV (Fig. 5). The end result is that the experiment has led the model cell to the resting membrane potential (\(\Delta V_m \approx -90.0\) mV) and places it at stationary equilibrium. At this point, \(\Delta V_m\) does not change any further, since the sum of all ionic current across the membrane is zero. In other words, the outward currents equal the inward currents: \(i_{\text{K}}(-\Delta\mu_{\text{K}}) + i_{\text{Na}}(\Delta\mu_{\text{Na}}) = -i_{\text{Na}}(-\Delta\mu_{\text{Na}}) - i_{\text{K}}(\Delta\mu_{\text{K}})\). Na\(^+\) and K\(^+\) chemical potentials are also constant because the pump exactly counteracts the leakage of these ions down their \(\Delta\mu\), making the cell, at the stationary state, effectively impermeable to those ions at the expense of metabolic energy.

**Final Considerations**

The four steps to bioelectrogenesis presented above describe the general modus operandi of the ECF-membrane-ICF system, i.e., the primary role of membrane capacitance, electrochemical potentials, and relative conductances of the membrane. However, as already mentioned, \(\Delta V_m\) ranges from \(-5\) to \(-100\) mV depending on the type of cell, demonstrating a large functional diversity of the cell membranes. To address this variability, we have to keep in mind the modus operandi of the system and look at the determinant factors of \(\Delta V_m\), i.e., the variables of the GHK equation: ion equilibrium potential (\(E_{\text{ion}}\)) and ion conductance (\(g_{\text{ion}}\)). Let us consider the following:

- First, according to the Nernst equation, \(E_{\text{ion}}\) is a function of intracellular ion concentration ([ion]\(_i\))/extracellular ion concentration ([ion]\(_e\)). Note, however, that while the homoeostatic systems of the body maintain the same [ion]\(_o\) for most cells, [ion]\(_o\) depends on each cell’s individual work. For Na\(^+\) and K\(^+\), the relatively low [Na\(^+\)]\(_i\) and high [K\(^+\)]\(_i\) are a function of Na\(^+\)-K\(^+\)-ATPase activity, which does not have the same molecular structure in every cell. Na\(^+\)-K\(^+\)-ATPase is an equimolar \(\alpha\)-\(\beta\) dimer assembly from distinct isoforms (four \(\alpha\) and three \(\beta\)) (5). The various combinations of \(\alpha\)-\(\beta\) complexes are tissue specific and present different sensitivity to regulating factors such as [Na\(^+\)]\(_i\)/[K\(^+\)]\(_l\) ion ratio, pH, intracellular ATP concentration, \(\Delta V_m\) and several circulating hormones (3, 5, 18), conferring wide functional variability to the pump. The number of pumps, which is a determinant factor for [Na\(^+\)]\(_i\) and [K\(^+\)]\(_i\) also varies largely in different tissues (3). Furthermore, [Na\(^+\)]\(_i\) and [K\(^+\)]\(_i\) also depend on the number of Na\(^+\) and K\(^+\) leakage channels and on the several secondary carrier proteins that use Na\(^+\) and K\(^+\) gradients, as the ions fluxes through them tend to cancel both gradients. Particularly, we can ask ourselves the following question: if the pump translocates more Na\(^+\) than K\(^+\), why is the gradient of Na\(^+\) smaller than the K\(^+\) gradient? This is because the distribution of Na\(^+\) and K\(^+\) is not an exclusive task of the pump; instead, several secondary transport systems use Na\(^+\) and K\(^+\) gradients as a driving force. Here, we can consider the Na\(^+\)/H\(^+\) exchanger, a secondary cotransporter ubiquitously expressed, that couples the inward flux of Na\(^+\) driven by \(\Delta\mu_{\text{Na}}\) to the extrusion of H\(^+\). The stoichiometry is 1Na\(^+\):1H\(^+\); therefore, it is electroneutral (15). Note that the Na\(^+\) influx decreases the Na\(^+\) gradient but does not interfere with the electrogentic capacity of the pump.

- Second, a given \(g_{\text{ion}}\) is not always due to a single molecular entity; instead, it is established by multiple channel types, each with distinct biophysical properties. Each type of cell expresses a particular set of channels, which work in concert to establish a specific value of \(\Delta V_m\). Moreover, the ion channels are not rigid structures; the open probability and number of the channels can be influenced by multiple factors like pH, temperature, voltage, and chemical mediators, introducing a high degree of complexity in the regulation of \(\Delta V_m\). Furthermore, in
many cells, $g_{\text{ion}}$ is not only represented by $g_{K^+}$ and $g_{Na^+}$; other conductances, which should be included in the GHK equation, may also contribute to $\Delta V_m$. Particularly, many neurons extrude $Cl^-$ through $K^+-Cl^-$ cotransporter-2. This secondary active sympmor establishes an electrochemical gradient for $Cl^-$, i.e., $E_{Cl}$ away from $\Delta V_m$ (23), which makes $Cl^-$ conductance a potential contributor to $\Delta V_m$. However, the influence of $Cl^-$ can also be quite complex due to the wide variation in the expression of different $Cl^-$ transporters and channels.

- Third, resting $g_{K^+}$, first postulated by Hodgkin and Huxley (9), is represented by the most diverse group of ion channels. However, it is thought that the primary contributors to $\Delta V_m$ fall into two main structural classes of channels: the one-pore domain, which encodes inward rectifier $K^+$ ($K_p$) channels (1, 11, 20), and two pore domains, which encode two-pore $K^+$ ($K_{2p}$) channels (1, 20). Each class of $K^+$ channel encompasses ~15 different members with distinct biophysical properties.

- Fourth, resting $g_{Na^+}$, which was also described by Hodgkin and Huxley (8), is also underlined by multiples types of channels. However, it seems that the $Na^+$ leak channel nonselective (NALCN) channel is the main contributor that counterbalances $K^+$ leakage (19). Surprisingly and as its name suggests, the NALCN channel is not selective for $Na^+$; instead, it is a cation-selective channel (19).

- Finally, we also have to consider the direct contribution of the $Na^+-K^+$ pump to $\Delta V_m$. Following Ohm’s law, this contribution depends on the amplitude of the current established by the pump activity and the resistance of the membrane. Accordingly, the resulting $\Delta V_m$ varies in different types of cells. The small contribution of the $Na^+-K^+$ pump to resting membrane potential, as ~2.5 mV in the model cell, is revealed with the inhibition of the pump by specific drugs, which typically results in a small depolarizing range from ~0.45 mV (13) up to ~10 mV (7).

In summary, the value of $\Delta V_m$ is established by a particular set of ion channels and transporters, of molecular compositions and concentrations specific to the different types of cells, that can also show large variability at different physiological and pathophysiological conditions. To help the students have a first set of ion channels and transporters, of molecular compositions assume that since the ICF and ECF are at osmotic equilibrium (chemical potential of intracellular cations = chemical potential of extracellular cations), if $\Delta V_m = 0$, there will be no $U$ and, therefore, no current through NALCN channels]. Finally, the kinetics of the channels are quite varied. Particularly, at voltages that are depolarized compared with the resting membrane potential, $K_{ir}$ channels are blocked by intracellular $Mg^{2+}$ (and by other polyvalent cations), giving rise to the region of negative slope of the $K_{ir}$ current-voltage relationship (20).

Taking these points into consideration, we can see on the graph that at $\Delta V_m = 0$ (at the beginning of our experiment), and more so at $\Delta V_m = -2.5$ mV, when the pump has already built the gradients, the outward $I_{Na^+}$ carried by $K_{2p}$ channels is very large and drives the voltage toward $E_{K^+}$. At increasingly negative membrane potentials, $I_{Na^+}$ through $K_{2p}$ channels decreases as a function of ($\Delta V_m - E_{K^+}$), $K_{ir}$ channels lose $Mg^{2+}$ blockage, and inward current through NALCN channels increases progressively as a function of ($\Delta V_m$ = NALCN equilibrium potential). Note that at resting membrane potential, i.e., $\Delta V_m = -90.0$ mV, the $K^+$ currents carried by $K_{2p}$ and $K_{ir}$ channels are added and are counterbalanced by the current through NALCN channels. In the context of this article, we will consider NALCN channels permeable to $Na^+$ and $K^+$, so as $I_{Na^+}$ and $I_{K^+}$ are of opposite directions at $\Delta V_m = -90.0$ mV, the resultant inward current through NALCN channels observed at the resting membrane potential is carried by $Na^+$. Note then that the inward $I_{Na^+}$ through NALCN channels is 50% larger than the outward $I_{Na^+}$ ($K_{ir} + K_{2p}$ channels); both these currents are canceled by $Na^+\text{-}K^+$-ATPase, which generates outward $I_{Na^+}$ 50% larger than the inward $I_{K^+}$ keeping the cell at stationary equilibrium.
Discussion and Conclusions

The separation of charges across the cell membrane is a process of great elegance that sustains life and runs against entropy. It is very expensive for the cell, accounting for a large portion of the metabolic cost. To help approach this important issue, teachers and students have the support of several books on physiology and of some didactic models described in the literature (2, 17), which provide excellent teaching tools. However, to overcome my own difficulties in explaining it clearly and yielding positive outcomes, I have implemented an alternative model for teaching bioelectrogenesis based on a theoretical approach in the classroom. I believe that the strategy described here gives a broader view and clearly identifies and quantifies the various mechanisms involved in bioelectrogenesis. I hope that it may help others in the process of teaching and learning the basis for the generation of resting membrane potential.

To develop the model, I considered the concept of cumulative learning, in which previously acquired knowledge functions as building blocks for new learning (6, 12, 16). In this sense, I first point out in Initial Considerations some review concepts upon which the bioelectrogenesis is built. In the classroom, I first present those basic concepts while the students are encouraged to a multidisciplinary approach to living systems. In fact, students have already acquired these concepts in high school; we only review and emphasize that biological structure is governed by the same physical and chemical laws that govern the universe as a whole. In addition, a number of authors (4, 14, 22) have highlighted the importance of instruction sequencing in learning activities, so that the order and organization of the content influence the processing and retention of information. Here, the model starts from a “reset cell” at baseline energy state and goes through all the basic processes toward an energized cell at stationary equilibrium, providing a simple-to-complex sequence of instruction in a constructivist strategy.

The key point of the model is the successive insertion of different proteins into the cell membrane, which brings into evidence the capacitive and resistive properties of the membrane and highlights that the cell and its surroundings are a system pushed out of thermodynamic equilibrium by a constant input of metabolic energy. In my experience, the use of the didactic model and the sequence of the topics described here provide an effective way of explaining and learning resting membrane potential. Students are easily engaged in logical reasoning and are taken through bioelectrogenesis in a coherent way, from the start (reset cell) to the end (energized cell). My perception is that the didactic model has a significant educational appeal; its design helps to match teaching with learning and allows students to qualitatively and quantitatively identify the role of each element involved in $\Delta V_m$ generation. Furthermore, this didactic model serves as a template for the teaching of the action potential as well as other common mechanisms such as electrotonic potentials and synaptic transmission. I must consider, however, that although the model can incorporate other elements such as $\text{Cl}^-$ and $\text{Ca}^{2+}$ channels as well as Donnan and border potentials, here I only analyzed the basic mechanisms as a first approach to bioelectrogenesis; it is an invitation, or an introductory session, for neurophysiology.

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