ILLUMINATIONS

Which way do the ions go? A graph-drawing exercise for understanding electrochemical gradients

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INFORMATION IS TRANSMITTED through the nervous system via changes in membrane potential (\(V_m\)). These changes occur when ions cross the cell membrane via ion channels. Each ion’s movement is governed by an electrochemical driving force (EDF) with two subcomponents: an electrical driving force (EDF) and a chemical or concentration driving force (CDF). Thus, to comprehend electrical signaling in the nervous system, a strong understanding of EDFs and their subcomponents is essential. Here I present a graph-drawing method by which undergraduate students can determine, at any given \(V_m\), whether a specific ion will flow inward or outward, and thus whether the cell will hyperpolarize or depolarize when that ion’s channels open. Through this method, conceptual lessons on EDFs may be modified to emphasize authentic problem-solving, as recommended by recent publications on reforming undergraduate physiology and biology education (1, 6).

ECDFs: general concepts vs. specific problems. Flow down gradients has been identified as one of the most important core concepts in all of physiology education (6, 7). This concept is especially prominent in neurophysiology lessons on the movement of ions through cell membranes, in which students learn that ions are simultaneously governed by EDFs and CDFs. This idea of “combining” two different types of gradients is a novel one for many undergraduates; however, most of them readily grasp that ions carry electrical charges and have chemical concentrations and, therefore, must be subject to both EDFs and CDFs. Moreover, illustrative examples are easy to come by. The typical “resting” neuron has a negative \(V_m\) (i.e., the inside of the cell is negative relative to the outside), a high intracellular K\(^+\) concentration, and a high extracellular Na\(^+\) concentration. These basic facts can be illustrated in a simple drawing of a neuron (or a generic cell) with minus signs on the inside of the membrane, plus signs on the outside, a big “K” for K\(^+\) ions, and a big “Na” for Na\(^+\) ions.

After introducing this material conceptually, most physiology courses then approach it quantitatively via the Nernst equation. As devised by German scientist Walther Nernst, this equation calculates a given ion’s equilibrium potential (\(E_{ion}\)) based on the ion’s charge and the ion’s extracellular and intracellular concentrations (2). It can be written in various forms, such as the following (for a temperature of 20°C):

\[
E_{ion} = \frac{58 \text{ mV}}{z} \times \log_{10} \left( \frac{[\text{ion}]_{\text{out}}}{[\text{ion}]_{\text{in}}} \right)
\]

where \(z\) is the ion’s valence (e.g., +2 for Ca\(^{2+}\), −1 for Cl\(^−\)) and \([\text{ion}]_{\text{out}}\) and \([\text{ion}]_{\text{in}}\) are the ion’s extracellular and intracellular concentrations, respectively. For our purposes, the key point about the Nernst equation is that it solves for the EDF that exactly counterbalances an ion’s CDF, such that there is no net EDF and no net movement of the ion into or out of the neuron (i.e., the ion is at equilibrium).

The Nernst equation is a useful tool for solving neurophysiology problems. In particular, it can be used to determine whether any given ion will flow inward or outward at a given \(V_m\) and thus whether its flux will depolarize or hyperpolarize the neuron, and thus increase or decrease the probability of an action potential, the fundamental unit of neural signal transmission. Regrettably, many introductory physiology courses stop just short of formally teaching students this application of the Nernst equation (i.e., to solve practical problems on how the opening of specific ion channels affects neural signaling).

Consider the following example. If a neurotransmitter such as GABA or glycine opens chloride (Cl\(^−\)) channels on a postsynaptic neuron (extracellular Cl\(^−\) concentrations \([\text{Cl}^-]_{\text{out}} = 110 \text{ mM}\); intracellular Cl\(^−\) concentrations \([\text{Cl}^-]_{\text{in}} = 5 \text{ mM}\;V_m = −65 \text{ mV\; temperature = 20°C}\), will that neuron depolarize or hyperpolarize? Most instructors would agree that this type of problem covers an important fundamental issue in neural signaling, and that it is desirable for our students to be able to solve it. And most students, on seeing the problem, realize that the Nernst equation is relevant and plug in the appropriate values to get a Cl\(^−\) equilibrium potential of −78 mV. But many of them, nevertheless, are stumped by the bottom line question of whether Cl\(^−\) will flow inward or outward.

Moving toward solutions. As an instructor teaching this material in the third quarter of an introductory biology series for biology majors, I was frustrated by this impasse. My students had all of the information they needed to solve meaningful problems, but they could not quite put it all together. How could I help them across the finish line? How could they move from being conversant in the general concepts of EDFs to solving specific problems about them?
My first attempt to help students solve these problems was based on the following key ideas.

- As stated above, $E_{ion}$ is the unique $V_m$ where the CDF and EDF are exactly counterbalanced.
- On a number line (one-dimensional graph) of possible $V_m$ values, the CDF will “overrule” the EDF (i.e., the ions will flow in a direction consistent with the CDF) at $V_m$ values on one side of $E_{ion}$; conversely, the EDF will “overrule” the CDF at $V_m$ values on the opposite side of $E_{ion}$.
- Therefore, solving problems like the one above is basically a matter of figuring out which side is which.

In explaining this to students, I noted that the EDF would trump the CDF at “extreme” $V_m$ values beyond the $E_{ion}$ (e.g., $V_m$ values even more negative than the $E_{ion}$ of $-78 \text{ mV}$, in the example above) and, therefore, that the CDF must trump the EDF on the opposite side of the $E_{ion}$. This line of reasoning was confusing for many students, who did not necessarily understand what I meant by “extreme” $V_m$ values. I then realized that a step-by-step protocol might make the above reasoning easier to follow and, consequently, devised the method below and used it in four physiology classes.

In addition to the points above, the method below employs one additional critical fact. At a $V_m$ of $0 \text{ mV}$, EDF is also zero, and ECDF is, therefore, determined solely by CDF. For the current example, the chemical gradient ([Cl$^-$/H$^+$]$_{out}$ = 110 mM, [Cl$^-$/H$^+$]$_{in}$ = 5 mM) will clearly drive Cl$^-$ into the cell. In general, by considering the point where $V_m = 0 \text{ mV}$, along with the point where $V_m = E_{ion}$, we can generate a graph that covers all possible $V_m$ values, as we shall see next.

**Fig. 1. A five-step method for predicting an ion’s direction of flux (into or out of a cell).**

### Table

<table>
<thead>
<tr>
<th>STEP</th>
<th>EXAMPLE: [Cl$^-$/H$^+$]$<em>{out} = 110 \text{ mM}$, [Cl$^-$/H$^+$]$</em>{in} = 5 \text{ mM}$, $20^\circ \text{C}$</th>
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<tbody>
<tr>
<td>(A)</td>
<td>Find the ion’s equilibrium potential ($E_{ion}$), if not provided.</td>
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<td></td>
<td>$E_{Cl} = \frac{59 \text{ mV}}{2} \times \log_{10} \left( \frac{[\text{Cl}^-]<em>{out}}{[\text{Cl}^-]</em>{in}} \right) = \frac{59 \text{ mV}}{2} \times \log_{10} \left( \frac{110 \text{ mM}}{5 \text{ mM}} \right) = -78 \text{ mV} $</td>
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<tr>
<td>(B)</td>
<td>Set up a graph with membrane potential ($V_m$) equivalent to EDF on the X axis and electrochemical driving force (ECDF) on the Y axis.</td>
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<td>(C)</td>
<td>Plot the X-intercept (where $Y = 0$), i.e., the point where $V_m = E_{ion}$.</td>
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<tr>
<td>(D)</td>
<td>Plot the Y-intercept (where $X = 0$), i.e., the point where $V_m = 0$. Here, since [Cl$^-$/H$^+$]$<em>{out} &gt; [\text{Cl}^-]/[\text{H}^+]</em>{in}$, the ECDF must be inward (gray region). While the point must be on the “into cell” arm of the Y axis, its exact location is arbitrary.</td>
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<tr>
<td>(E)</td>
<td>Connect the 2 points with a line. Here, the line shows that the ECDF is outward (white region) at $V_m$'s more negative than $-78 \text{ mV}$ and is inward (gray region) at $V_m$'s more positive than $-78 \text{ mV}$.</td>
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</table>
A. Find the $E_{ion}$, if not provided.
B. Set up a graph with $V_m$ (equivalent to EDF) on the x-axis and ECDF on the y-axis.
C. Plot the $X$-intercept (where $Y = 0$), i.e., the point where $V_m = E_{ion}$.
D. Plot the $Y$-intercept (where $X = 0$), i.e., the point where $V_m = 0$.
E. Connect the two points with a line.

A few explanatory notes may be useful here. First of all, the choice of axes in step B can be justified as follows. For any initial condition of intracellular and extracellular ion concentrations, a relatively small flux of ions across the cell membrane dramatically changes $V_m$ (and thus EDF) without appreciably altering the extracellular and intracellular concentrations (3). Therefore, the graph treats $V_m$ (and thus EDF) as variable, while allowing CDF to be constant. As generated by this method, ECDF-$V_m$ curves are reminiscent of the current-voltage curves that are ubiquitous in advanced neurophysiology (4), with ECDF occupying the y-axis instead of current.

By definition, ECDF = 0 at an $E_{ion}$; therefore, the line representing ECDF as a function of $V_m$ will always cross the x-axis where $V_m = E_{ion}$. But does the line slope upward or downward? This is equivalent to asking which driving force (CDF or EDF) “wins” on which side of $E_{ion}$. To find out, consider the point where $V_m = 0$ (and thus EDF = 0), and the direction of the flux depends only on the CDF (step D). If the chemical gradient drives the ion inward, as it does for Cl$^-$, this point can be placed on the “in” arm of the y-axis. A line through this point and the $E_{ion}$ point (step E) then shows that the CDF (inward) “wins” for $V_m$ values less negative than $-78$ mV, including the $V_m$ of $-65$ mV mentioned in the problem above, whereas the EDF (outward) “wins” for $V_m$ values more negative than $-78$ mV.

The finished graph may also be annotated as shown at the bottom of Fig. 1, step E. The possible $V_m$ values may be divided into three ranges: one where the strength of the CDF exceeds that of the EDF, one where the strength of the ECF exceeds that of the CDF, and one where both drive the ion in the same direction. The labeling of these three ranges (which will exist for any ion with a nonzero equilibrium potential) underscores the fact that CDF and EDF may act in cooperation with each other or in opposition to each other, depending on the $V_m$.

Comparison with Nolan. The problem of predicting ions’ direction of flux was addressed in one of the early volumes of this journal. Nolan (8) explained how the Nernst equation can be used to calculate chemical potentials (in millivolts) that can then be compared with electrical potentials (also in millivolts) to determine flux direction in cases where the CDF and EDF are in opposite directions. Nolan thus helps instructors give their students a thorough grounding in ECDFs. However, as I understand it, Nolan’s approach treats each possible $V_m$ as a separate problem for which CDF and EDF are calculated. Thus the present method may be considered an alternative to Nolan that graphically portrays the “big picture” of the relationship between $V_m$ and ECDF.

Because my graph-drawing method is designed to be quick and simple, it is less mathematical than that of Nolan, which might be a strength or a liability, depending on one’s students and teaching goals. In particular, note that the ECDF axis (the y-axis in Fig. 1) has no numbers or units; its arms are simply labeled “into cell” and “out of cell.” Also, a line is drawn through the two points (step E) without careful consideration of the line’s precise shape (e.g., linear vs. curvilinear). Instructors who dislike such simplifications may prefer to teach this material in the style of Nolan.

Whether one prefers the present approach or that of Nolan or another alternative, it is easy to create problems on predicting the direction of an ion’s flux. Simply choose an ion, list its intracellular and extracellular concentrations, and ask about the direction of flux at one or more $V_m$ values. In testing for true understanding, one need not be limited to physiologically realistic situations; Fig. 2 provides an example of a fanciful yet fair question.

Feedback on the method. Several biology and biochemistry faculty have considered the method presented here and have discussed it with me. In general, they agreed that having students determine directions of ion flux is a worthwhile goal, and that the steps in Fig. 1 give students a clear, helpful path toward this goal. A second theme of their feedback was that, even though I pitched my method as a simple alternative to more complex calculations (8), my approach might be best as a complement to, rather than a replacement for, these other exercises. One colleague noted that, since different explanations resonate with different students, showing them my method alongside that of Nolan (8) might actually be useful rather than redundant.

A final point made by colleagues was that students need tools beyond the present method for understanding aspects of neural signaling beyond ECDFs, such as resistance to flux (i.e., through ion channels) and the combined impact of multiple ions. Perhaps most obviously, $V_m$ can be calculated from the

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**Fig. 2.** An example of a test question that asks students to apply their understanding of ECDFs to a novel situation.
Goldman-Hodgkin-Katz equation. Students might also be prompted to consider nonselective cation channels, such as the acetylcholine receptors, on skeletal muscle cells and to contemplate why these channels mostly carry inward Na\(^{+}\) current rather than outward K\(^{+}\) current.

**Conclusion.** Solving meaningful problems should be a central part of physiology education (5) and is increasingly emphasized in efforts to reform biology education (1). The method presented here attempts to live up to this ideal by transforming a primarily conceptual treatment of ECDFs into a problem-based one, where students predict ions’ directions of flux, thus deepening their understanding of a fundamental issue in neural signaling. The method appears appropriate for introductory physiology students with modest quantitative skills.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**AUTHOR CONTRIBUTIONS**

G.J.C. prepared figures; drafted manuscript; edited and revised manuscript; approved final version of manuscript.

**REFERENCES**