Adv Physiol Educ 41: 556–559, 2017;
doi:10.1152/advan.00111.2017.

ILLUMINATIONS

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

Gregory J. Crowther
Division of Biological Sciences, School of STEM, University of Washington Bothell, Bothell, Washington

Submitted 7 August 2017; accepted in final form 15 September 2017

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 (ECDF) 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 ECDFs 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 ECDFs 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 movements 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^+" on the inside, and a big "Na^+" on the outside. Students can then see that Na^+ is driven into the cell by both its CDF and the EDF, whereas K^+ is driven inward by the EDF, but outward by its CDF. They can then reason that the actual direction of movement of K^+, and thus its effect on V_m, must depend on the relative magnitudes of the two driving forces. If they find out that K^+ flows out of the cell, they can conclude that the outward CDF is larger than the inward EDF.

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):

$$E_{ion} = \frac{58\ \text{mV}}{z} \times \log_{10}\left(\frac{[\text{ion}]_{out}}{[\text{ion}]_{in}}\right) \qquad (1)$$

where z is the ion's valence (e.g., $+2$ for Ca^{2+}, -1 for Cl^-) and $[ion]_{out}$ and $[ion]_{in}$ 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 ECDF 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 $[Cl^-]_{out} = 110$ mM; intracellular Cl^- concentrations $[Cl^-]_{in} = 5$ mM, $V_m = -65$ 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 ECDFs to solving specific problems about them?

Address for reprint requests and other correspondence: G. J. Crowther, Biological Sciences, School of STEM, University of Washington Bothell, Box 358538, Bothell, WA 98011 (e-mail: crowther@u.washington.edu).

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 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 mV, EDF is also zero, and ECDF is, therefore, determined solely by CDF. For the current example, the chemical gradient ($[Cl^-]_{out}$ = 110 mM, $[Cl^-]_{in}$ = 5 mM) will clearly drive Cl^- into the cell. In general, by considering the point where $V_m = 0$ 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.

Drawing a graph of ECDF vs. V_m. The above reasoning can be organized into a five-step method for students to follow. The details are shown in Fig. 1; the essence of the method is the following:

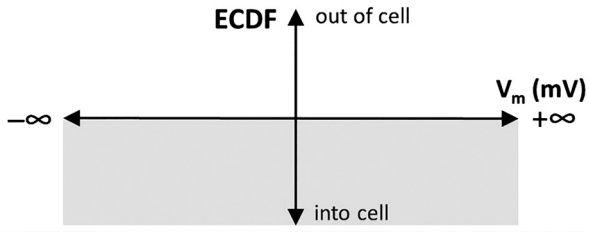
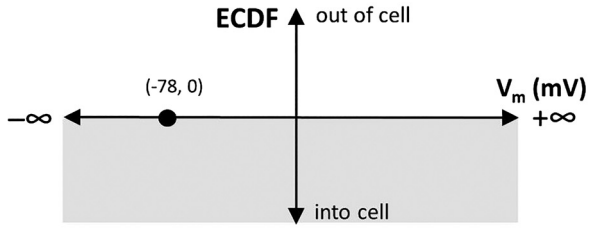
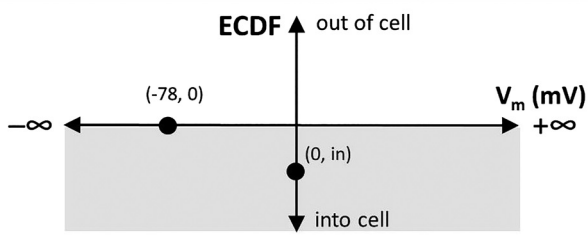
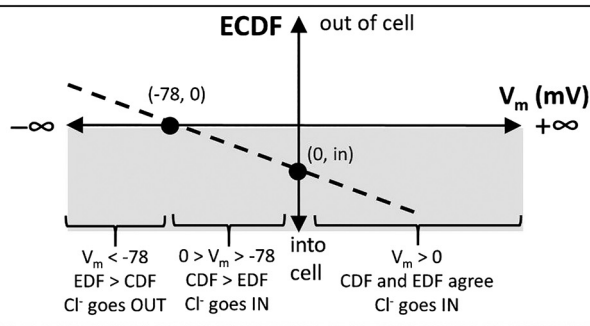
STEP	EXAMPLE: $[Cl^-]_{out}$ = 110 mM, $[Cl^-]_{in}$ = 5 mM, 20° C
(A) Find the ion's equilibrium potential (E_{ion}), if not provided.	$E_{Cl} = \frac{58\ mV}{z} * log_{10}\left(\frac{[Cl]_{out}}{[Cl]_{in}}\right) = \frac{58\ mV}{-1} * log_{10}\left(\frac{110\ mM}{5\ mM}\right) = -78\ mV$
(B) Set up a graph with membrane potential (V_m, equivalent to EDF) on the X axis and electrochemical driving force (ECDF) on the Y axis.	
(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. Here, since $[Cl^-]_{out} > [Cl^-]_{in}$, 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.	
(E) 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 mV and is inward (gray region) at V_m's more positive than -78 mV.	

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

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 assuming 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

Imagine an alien animal with neurons like ours except with different ions, different ion channels, and a resting membrane potential of -100 mV. The Nernst equation still holds true. Ion X^{2+} is at a concentration of 10 mM inside the cell and 100 mM outside the cell. Fill in each empty box of the following chart with INTO CELL, OUT OF CELL, or NEITHER.

Membrane potential	Direction X^{2+} is driven, considering only the electrical gradient	Direction X^{2+} is driven, considering only the chemical gradient	Direction X^{2+} is driven, considering the overall electrochemical gradient
-100 mV			
-58 mV			
-29 mV			
0 mV			
29 mV			
58 mV			
100 mV			

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.

ACKNOWLEDGMENTS

The following people provided constructive feedback on the method described here: Dr. Jennifer Breckler of San Francisco State University; and Drs. Salwa Al-Noori, Lori Robins, and Douglas Wacker of UW-Bothell.

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

1. **American Association for the Advancement of Science.** *Vision and Change in Undergraduate Biology Education: A Call to Action. A Summary of Recommendations Made at a National Conference Organized by the American Association for the Advancement of Science, July 15–17, 2009*. Washington, DC: AAAS, 2011.
2. **Funke K.** Solid state ionics: from Michael Faraday to green energy—the European dimension. *Sci Technol Adv Mater* 14: 043502, 2013. doi: 10.1088/1468-6996/14/4/043502.
3. **Hille B.** *Ion Channels of Excitable Membranes* (3rd Ed.). Sunderland, MA: Sinauer Associates, 2001.
4. **Kay AR.** What gets a cell excited? Kinky curves. *Adv Physiol Educ* 38: 376–380, 2014. doi:10.1152/advan.00039.2014.
5. **Michael J.** The Claude Bernard Distinguished Lecture. In pursuit of meaningful learning. *Adv Physiol Educ* 25: 145–158, 2001.
6. **Michael J, Cliff W, McFarland J, Modell H, Wright A.** *The Core Concepts of Physiology: A New Paradigm for Teaching Physiology*. New York: Springer-Verlag, 2017. doi:10.1007/978-1-4939-6909-8.
7. **Michael J, McFarland J.** The core principles ("big ideas") of physiology: results of faculty surveys. *Adv Physiol Educ* 35: 336–341, 2011. doi: 10.1152/advan.00004.2011.
8. **Nolan WF.** A problem-solving approach to teaching electrochemical driving force to undergraduates. *Am J Physiol* 259: S1–S3, 1990.