A classic experiment revisited: membrane permeability changes during the action potential

Carolyn L. Powell1 and Angus M. Brown1,2
1School of Life Sciences, University of Nottingham, Nottingham, United Kingdom and 2Department of Neurology, University of Washington, Seattle, Washington

Abstract

The ability to understand the relationship between the reversal potential and the membrane potential is a fundamental skill that must be mastered by students studying membrane excitability. To clarify this relationship, we have reframed a classic experiment carried out by Hodgkin and Katz, where we compare graphically the membrane potential at three phases of the action potential (resting potential, action potential peak, and afterhyperpolarization) to reversal potential for K+ (EK), reversal potential for Na+ (ENa), and membrane potential (EM) (calculated by the Goldman Hodgkin Katz equation) to illustrate that the membrane potential approaches the reversal potential of the ion to which it is most permeable at that instant.

action potential; GHK equation; Nernst equation; membrane potential; permeability

The concept of the resting membrane potential can appear daunting to novice students, presenting a considerable impediment to understanding membrane excitability. In this brief illuminations article, we suggest an approach for teachers that encourages student understanding of the relationship between the Nernst equation, the Goldman Hodgkin Katz (GHK) voltage equation and the membrane potential. By initially assuming the membrane is permeable to only one ion (K+), the membrane potential is equal to the reversal potential for K+ (EK) and can be calculated using the Nernst equation. Four articles have recently been published in this journal, which contain the requisite information on the Nernst equation (1–4). Teachers should stress the importance of student ability to (1) predict the direction of K+ movement when the membrane potential does not equal EK (3); (2) understand that when intracellular K+ concentration ([K+]i) is constant and extracellular K+ concentration ([K+]o) increases by an order of magnitude, at mammalian body temperature the membrane potential will depolarize by 61.5 mV (4); and (3) calculate the unknown parameter when two of the three parameters in the Nernst equation are known (1).

When the Nernst equation has been mastered, teachers can progress to describe the GHK voltage equation, an extension of the Nernst equation that predicts the membrane potential of a cell membrane permeable to more than one ion, as occurs in neurons (5): astrocytes are a unique example of a cell whose membrane is exclusively permeable to K+ (6). An important consequence of this is that in astrocytes at rest there is no net flux of K+, but in neurons at rest there is significant flux of Na+ and K+ (7), where the membrane potential is determined by the combined movement of multiple currents. The magnitude of the contribution of each current is dependent on the ion concentration gradient across the membrane and the permeability of the membrane to each ion. Students should be reassured that it is usually sufficient to make reasonable estimates of membrane potential based on understanding of the GHK voltage equation, rather than calculating its exact value (8).

The application of the Nernst equation to estimate the potential difference across semipermeable cell membranes (9) was a major advance in the field of physiology, as it allowed predictions of I) the polarity of the resting membrane potential before the introduction of intracellular recording techniques enabled its measurement (10), and 2) membrane ionic permeability based on comparisons between the membrane potential and reversal potentials (5). The Nernst equation determines a potential difference, referred to as the reversal potential, which is dependent on the temperature and the ratio of the intracellular and extracellular ion concentrations (1–4). At the reversal potential the net driving force on the ion is zero, i.e., the chemical gradient driving the ions in one direction is matched equally by the electrical gradient driving ions in the other direction and no net current flows across the membrane (2, 3). Thus for cells permeable to only one ion the reversal potential is equal to the membrane potential, which allows quantitative estimates of the membrane potential response to a variety of physiological processes such as increased neural activity (11). Only one type of cell, the astrocyte, is known to behave in this way (12), limiting the application of the Nernst equation. This was addressed when Hodgkin and Katz adapted the theories of Goldman, which
described the movement of ions across a membrane, to derive the GHK voltage equation (13), which is an extension of the Nernst equation that takes into account that most membranes are permeable to more than one type of ion (5). In this brief article we demonstrate how application of the Nernst equation and GHK voltage equation can illuminate a classic experiment carried out by Hodgkin and Katz (10, 13). We consider a membrane that is, for the sake of clarity, permeable to only two ions, Na$^+$ and K$^+$. If the intra- and extracellular ion concentrations are known, the GHK voltage equation can be used for two main purposes, I) to estimate the relative permeabilities of Na$^+$ and K$^+$ if the membrane potential is known (Fig. 1), or 2) to calculate the membrane potential if the relative permeabilities of Na$^+$ and K$^+$ are known (Fig. 2) (8).

The GHK voltage equation can be expressed as:

$$E_m = 55 \log_{10} \left( \frac{P_K [K^+]_o + P_{Na} [Na^+]_o}{P_K [K^+]_i + P_{Na} [Na^+]_i} \right) \quad (Eq. 1)$$

at seawater temperature, where $E_m$ is the membrane potential, $P_K$ is the permeability of K$^+$ relative to Na$^+$ and is usually taken as 1, and $P_{Na}$ is the permeability of Na$^+$ relative to K$^+$. This is a useful expression as the Hodgkin, Huxley, and Keynes’s calculations showed that the transmembrane ion potentials are identical as to be considered negligible, i.e., the ion concentrations did not change following activity (14, 15). For squid axon, these concentrations can be taken as: [K$^+$]$_i = 345$ mM, [K$^+$]$_o = 10$ mM, [Na$^+$]$_i = 72$ mM and [Na$^+$]$_o = 450$ mM at rest (16) with $E_{Na} = 52$ mV and $E_K = -88$ mV, where $E_{Na}$ is Na$^+$ reversal potential and [Na$^+$]$_i$ and [Na$^+$]$_o$ are the intracellular and extracellular Na$^+$ concentrations.

**Figure 1.** Relative permeability to Na$^+$ and K$^+$ during the action potential. A: the simulated action potential. The components of the action potential are identified as i) the resting membrane potential, ii) the action potential peak, and iii) the afterhyperpolarization (AHP). Horizontal lines indicate the values of reversal potential for K$^+$ and Na$^+$ ($E_{Na}$ and $E_K$). B: $P_{Na}$ (relative to $P_K$ of 1) during the action potential calculated according to Eq. 1 using Microsoft SOLVER (red line and right axis), where $P_K$ is the permeability of K$^+$ relative to Na$^+$ and is usually taken as 1 and $P_{Na}$ is the permeability of Na$^+$ relative to K$^+$. Note how as the membrane potential depolarizes toward threshold the value of $P_{Na}$ is close to 0. $P_K$ (relative to $P_{Na}$ of 1) is high at rest and decreased during the action potential and then increased to its maximal value at the peak of the AHP (blue line and left axis: note log scale).

**Figure 2.** Effect of extracellular K$^+$ concentration ([K$^+$]$_o$) on action potential profile. A: 4 action potentials simulated under various [K$^+$]$_o$: 5 mM [K$^+$]$_o$ (blue), 10 mM [K$^+$]$_o$ (green), 15 mM [K$^+$]$_o$ (yellow), and 20 mM [K$^+$]$_o$ (red). B: the values of reversal potential for K$^+$ ($E_K$), reversal potential for Na$^+$ ($E_{Na}$), and membrane potential ($E_m$) calculated for [K$^+$]$_o$ over the range 1 to 1,000 mM. The color-coded vertical lines indicate [K$^+$]$_o$ for equivalent conditions under which the traces in A were simulated. The intercept of these lines with $E_m$, $E_{Na}$, and $E_K$ approximate to the value of the resting membrane potential, action potential peak, and afterhyperpolarization peak is shown, respectively.

**A GUIDE TO INTERPRETING THE FIGURES**

$P_K$ and $P_{Na}$ can be calculated during an action potential using the Microsoft SOLVER function (17) applied to Eq. 1, where the individual components of the action potential profile are identified as I) the resting membrane potential, 2) the action potential peak, and 3) the afterhyperpolarization (AHP) (Fig. 1A). When $P_{Na}$ is calculated relative to a $P_K$ of 1, its value is low at rest (0.025). Application of a stimulus depolarizes the membrane potential, which approaches threshold (~50 mV) but $P_{Na}$ remains low, consistent with the critical point made by Hodgkin that it is local circuit currents that depolarize the membrane potential toward threshold (18, 19). As the membrane potential depolarizes, $P_{Na}$ increases and Na$^+$ becomes the dominant ion in determining membrane potential.
potential. At the peak of the action potential, the membrane potential approaches $E_{\text{Na}}$ and $E_{\text{K}}$ to gain a more complete picture of the permeability changes that underlie the action potential. We have used a simulation of the Hodgkin Huxley squid axon (20) to reproduce a classic experiment carried out by Hodgkin and Katz in which action potentials were recorded in the presence of various $[K^+]_o$ (Fig. 2A). Hodgkin and Katz sought to identify which component of the action potential $Na^+$ and $K^+$ contributed by independently reducing the concentration of the ions bathing the squid axon and then assessing which component(s) of the action potential were affected. They deduced that $Na^+$ contributes to the upstroke of the action potential but the $K^+$ contribution was subtler and affected the resting membrane potential, the rate of repolarization and the magnitude of the AHP. The resting $[K^+]_o$ was either 5 mM, 10 mM, 15 mM, or 20 mM with equimolar substitution of $Na^+$ such that $[K^+]_o + [Na^+]_o = 460$ mM. The main effects of this experiment were 1) as $[K^+]_o$ decreased, the resting potential hyperpolarized slightly; 2) as $[K^+]_o$ decreased, the action potential peak was unchanged; and 3) as $[K^+]_o$ decreased, the AHP amplitude significantly increased. These three effects can be satisfactorily explained by referring to each action potential component relative to $E_m$, $E_{\text{Na}}$, or $E_K$. When plotted against $[K^+]_o$ on a log base 10 scale, $E_K$ [calculated as $5 \log_{10}[[K^+]_o/345]]$ is a straight line with a slope of 55 mV, whereas $E_{\text{Na}}$ [calculated as $5 \log_{10}([460 - [K^+]_o]/72)]$ commences as a horizontal line at low $[K^+]_o$ and then hyperpolarizes dramatically as $[K^+]_o$ increases. The membrane potential ($E_m$) is calculated according to Eq. 1 with a $P_{\text{Na}}$ value of 0.04. The resting membrane potential of neurons and astrocytes can be modeled as $E_m$ and $E_K$, respectively, and illustrates the key point that the rate at which the membrane potential depolarizes with increased $[K^+]_o$ within the physiological range (3–12 mM) is greater in astrocytes than neurons, making them ideal sensors of increased neuronal activity (21). If a vertical line is drawn from each of the four values of $[K^+]_o$ in the simulation (Fig. 2B), the intercept with $E_{\text{Na}}, E_{\text{Na}}$, and $E_K$ provides a good estimate of 1) the resting membrane potential, 2) the action potential peak, and 3) the magnitude of the AHP, respectively. This suggests the membrane is predominantly permeable to $K^+$, but has a small finite $Na^+$ permeability, at rest, but is predominantly permeable to $Na^+$ at the peak of the action potential and to $K^+$ at the AHP peak. In conclusion the membrane potential approaches the reversal potential of the ion to which it is most permeable at that instant.

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

A.M.B. conceived and designed research; A.M.B. performed experiments; C.L.P. and A.M.B. analyzed data; A.M.B. interpreted results of experiments; C.L.P. and A.M.B. prepared figures; A.M.B. drafted manuscript; A.M.B. edited and revised manuscript; C.L.P. and A.M.B. approved final version of manuscript.

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