RESTING MEMBRANE POTENTIALS: A STUDENT TEST OF ALTERNATE HYPOTHESES

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The frog sartorius muscle is a model tissue for demonstrating to physiology students the principles underlying both membrane phenomena and hypothesis testing. Myocytes can be impaled with conventional glass microelectrodes to measure membrane voltage ($V_m$). Further, $V_m$ is observed as extracellular K+ is altered and a K+ channel blocker is added. After the experiment, students examine the underlying assumptions of the Nernst equilibrium and the Goldman-Hodgkin-Katz equation. They ultimately determine which of the two algorithms best predicts the measured $V_m$. In addition, students learn micromanipulation and impalement techniques. This experiment facilitates the student’s understanding of membrane permeability, ionic gradients, and membrane voltage.

Keywords: physiology teaching; membrane potential; frog muscle; Nernst equilibrium equation; Goldman-Hodgkin-Katz equation

The potential difference across a biological membrane is a fundamental concept in physiology. Introductory courses usually commence with discussions of membrane structure, transport, and the development of membrane potentials (3). First, diffusion and equilibrium potentials are demonstrated using simple laboratory equipment and artificial membranes (1). Donnan and Nernst equilibrium (NE) potentials are discussed to illustrate the relationship between chemical and electrical driving forces (3, 10). However, as the discussion moves toward biological membranes, permeability, and voltage ($V_m$), students easily become confused in the absence of a concrete learning experience. Because undergraduates enroll in these courses with minimum preparation, abstract biophysical concepts such as electromotive force and $V_m$ strain their intellectual comfort. This unfortunate circumstance easily results when departments move a large number of students through a popular biomedical curriculum. Although a number of computer simulations are available to model membrane potential (2, 9), these tutorials alone do not seem to resolve the intricacies of the concept for students. However, if a theoretical construct can be coupled tightly to a practical experience, there is an increased probability that a fundamental tenet will be learned.

I have developed an educational laboratory protocol that introduces new physiology students to the principles of bioelectricity through progressive stages of discovery. To understand the roles of the NE and Goldman-Hodgkin-Katz (GHK) equations (4, 6) in predicting $V_m$, the frog sartorius muscle can be impaled and studied using conventional glass microelectrodes (5, 8). In this classic experiment, the $V_m$ can be altered by changing the ionic composition of the external bathing solution. In turn, the relationship between $V_m$ and the ionic concentration can be used to assess which mathematical algorithm (NE or GHK equation) more precisely estimates the $V_m$.

Over the last few years, the technology for microelectrode studies of the frog sartorius muscle has been...
used with great success in my undergraduate teaching laboratory. This paper describes an undergraduate laboratory exercise that couples the estimation of \( V_m \) by both the NE and the GHK equations with its direct measurement in myocytes. For reasons of cost and ease of use, I have adopted *The Membrane Potential Problem Solver* by R. A. Meiss (9) for use in my course. My students have used this program with immense satisfaction to estimate \( V_m \) by the GHK equation. After completing both the calculations and the measurements, students assess which algorithm will best describe the electrical behavior of the frog sartorius muscle membrane. Also, they are asked to enumerate the specific differences in the assumptions of the NE and GHK equations in retrospect. Through this exercise, students learn to test alternate hypotheses using empirical data. In addition, they gain practical experience in both micromanipulation and measurement of \( V_m \).

**MATERIALS AND METHODS**

*Rana pipiens*, purchased from Nasco (Ft. Atkinson, WI), are maintained in running well water and fed crickets every 2 wk. After double pithing, the sartorius muscle is removed from the ventral surface of each leg and rinsed in frog Ringer solution (in mM: 115 \( \text{Na}^+ \), 3 \( \text{K}^+ \), 2.7 \( \text{Ca}^{2+} \), 121.4 \( \text{Cl}^- \), 2.5 \( \text{HCO}_3^- \); osmolarity 210–230 osM). Carefully, the muscles are stretched to form a smooth rectangle with the medial surface turned up and pinned to the bottom of a finger bowl covered with a slanted layer of opaque wax. Various solutions can then be superfused over the preparation by gravity as excess fluid is removed by suction. Usually, only one muscle is used by each student group during the entire experiment. The muscle fibers are initially exposed to a frog Ringer solution containing 3.0 mM \( \text{K}^+ \) as they are impaled with a microelectrode (see **Added proof**). Subsequently, the muscle is washed sequentially for 10 min with Ringer solution containing either 10, 30, or 100 mM \( \text{K}^+ \). To maintain osmotic balance in these solutions, increases in KCl are matched by an equimolar reduction of the NaCl in the Ringer solution. After \( V_m \) is measured in the most concentrated \( \text{K}^+ \) solution, the muscle is returned to the 30 mM \( \text{K}^+ \) for 10 min. \( \text{BaCl}_2 \) is added to a final concentration of 10 mM. The experiment takes \( \sim 2.5 \) h of class time.

**Hardware requirements.** Glass microelectrodes are fabricated from fiber-filled borosilicate glass (1.2 mm OD, stock no. 1BBL, WPI, Sarasota, FL) with a Narishige horizontal pipette puller (PN-3; Pacer, Los Angeles, CA). After pulling, the pipettes are back-filled with 5.0 M KCl, and the tip resistance is measured using a Neuroprobe model 1600 electrometer (A-M Systems, Seattle, WA). Only those with a tip resistance between 20 and 30 MΩ are used for impalement. To measure \( V_m \), the pipette is connected to a voltage-follower amplifier (input impedance of 0.5 \( \times 10^{12} \) \( \Omega \), VF-11, Warner Instrument, Hamden, CT) and a Metex M-3800 digital multimeter (Jameco, Belmont, CA) using an Ag-AgCl reference electrode as ground. Electrode junctional potentials are reduced by electrically adjusting the tip voltage to “0” mV. The head stage of the amplifier and glass pipette is maneuvered using a Prior Student micromanipulator (Stoelting, Chicago, IL) and a magnetic stand on a 0.25 \( \times \) 12 \( \times \) 24-in. steel plate. The impalement apparatus is housed in a grounded wood/metal Faraday cage.

To impale myofibers, the pipette is guided by the manipulator as the tip is observed with a binocular dissecting microscope. When the pipette moves near the sarcolemma, the electrode voltage deflects from 0 to about –25 mV. Care is then taken to advance the pipette very slowly. This will produce an impalement or micropuncture. For an impalement to be considered reliable, \( V_m \) should fluctuate no more than ±2 mV for a period of not less than 2 min. The magnitude of the voltage should be near a value predicted by either the NE or GHK equation. As the microelectrode is withdrawn from the preparation into the bath, the junctional offset voltage varies no more than ±3 mV from 0 mV. If the tip potential changes significantly, the tip resistance should be measured again, and undesirable electrodes should be discarded. Students are encouraged to make five “good” impalments under each extracellular \( \text{K}^+ \) concentration ([\( \text{K}^+ \)]),.

**Predicting \( V_m \).** Two algorithms are used to calculate \( V_m \). If a barrier is selectively permeable to a single charged particle and the permeability is unaltered by the transmural voltage, the NE equation can be...
used to estimate $V_m$ as follows

$$V_m = \frac{RT}{zF} \ln \frac{n_1}{n_2} \text{ (volts)}$$

where $R$ is a gas constant (8.13 J/Kmol), $T$ is temperature in Kelvin, $z$ is valence, $F = 96,500$ C/mol equivalent, $n_1$ is the permeable ion in compartment 1, and $n_2$ is the permeable ion in compartment 2. For a monovalent ion, $ln$ can be converted to $log_{10}$ by multiplying by 2.3 and if $T$ is 25°C ($T = 298$ K), then

$$V_m = 0.058 \log \frac{n_1}{n_2} \text{ (volts)}$$

or

$$V_m = 58 \log \frac{n_1}{n_2} \text{ (mV)}$$

As an alternative, the GHK equation (2) can be used

$$V_m = \frac{RT}{zF} \ln \frac{[K]_o + P_{Na}/P_K [Na]_o}{[K]_i + P_{Na}/P_K [Na]_i} \text{ (volts)}$$

where $P$ is permeability, $i$ and $o$ are inside and outside, respectively, and brackets denote concentration. At room temperature

$$V_m = 58 \log \frac{[K]_o + P_{Na}/P_K [Na]_o}{[K]_i + P_{Na}/P_K [Na]_i} \text{ (mV)}$$

Software. To facilitate calculation of $V_m$ using the GHK equation, The Membrane Potential Problem Solver (9) was maintained on the hard drive of a MS-DOS-based 486 IBM-compatible computer with a VGA monitor and math coprocessor. For both NE and GHK equations, $[K^+]$, and $[Na^+]$, are assumed to be 125 and 4 mM, respectively (7). For the GHK equation, chloride is expected to exhibit a “Donnan” equilibrium with $[K^+]$ while the relative membrane permeabilities for $K^+$, $Cl^-$, and $Na^+$ are assumed to be 1, 0.50, and 0.04, respectively. When the GHK equation is used, it is important to recall that increases in $[K]_o$ were matched with equimolar reductions in $[Na]_o$. Consequently, two concentration changes must be simultaneously incorporated to calculate $V_m$.

CLASSROOM RESULTS

To begin the experiment, students must first learn to impale the fibers of sartorius muscle with care. The first 30 min of the laboratory are usually spent making new electrodes to replace broken ones. As students develop proficiency with the manipulator, the following question arises: “What voltage values are good?” Two criteria are used. 1) Using the permeabilities and $K^+$ and sodium concentrations given above, the students can calculate expected $V_m$ values using both the NE and GHK equations (Table 1). The $V_m$ is considered to be reliable if it falls in this range. 2) The electrode maintains a tip resistance between 20 and 30 MΩ after being withdrawn from a myocyte. If the $K^+$ solutions are administered by increasing concentration, the experiment usually proceeds very satisfactorily. At the end of the experiment, 10 mM Ba$^{2+}$ in a 30 mM $K^+$ superfusion solution is used as a noncompetitive inhibitor blocking $K^+$ channels. Figure 1 graphically compares the experimental results of the four classes from Table 1 with the predictions of the NE and GHK algorithms (see Added proof).

### TABLE 1

<table>
<thead>
<tr>
<th>$[K^+]_0$, mM</th>
<th>Prediction</th>
<th>Mean ± SD</th>
<th>Group Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>GHK</td>
<td>$G_1$</td>
</tr>
<tr>
<td>5</td>
<td>-93.9</td>
<td>-74.2</td>
<td>-72.4 ± 3.4</td>
</tr>
<tr>
<td>10</td>
<td>-63.6</td>
<td>-57.4</td>
<td>-59.3 ± 1.7</td>
</tr>
<tr>
<td>30</td>
<td>-35.9</td>
<td>-34.9</td>
<td>-38.4 ± 1.0</td>
</tr>
<tr>
<td>100</td>
<td>-5.6</td>
<td>-5.5</td>
<td>-11.9 ± 1.9</td>
</tr>
<tr>
<td>30 + 10 mM Ba$^{2+}$</td>
<td>+0</td>
<td>+0</td>
<td>-4.6 ± 4.6</td>
</tr>
</tbody>
</table>

Units are mV, $[K^+]_0$, extracellular $K^+$ concentration, $V_m$, membrane voltage, NE, Nernst equilibrium, GHK, Goldman-Hodgkin-Katz. G is class average with no. of impalements in parentheses.
FIG. 1. Membrane voltage ($V_m$) in frog sartorius muscle depolarized by elevating extracellular K⁺ concentration ($[K^+]_o$). Broken line, predicted by Nerst equation; solid line, predicted by Goldman-Hodgkin-Katz equation; •, measured $V_m$ (mean ± SE).

DISCUSSION

The logistics for this exercise are very manageable for classes of up to 16 students. I conduct this experiment by dividing the class into four groups and requiring each member to produce a satisfactory impalement. The equipment cost for this laboratory experiment is not prohibitive. A pipette puller ($2,000) and Neuroprobe ($1,900) were borrowed from a research laboratory. Fiber-filled borosilicate tubing costs about $40–60 per 500 pieces. The voltage-follower amplifier costs about $260, whereas the digital multimeter is $35. The micromanipulator and stand cost $250, and a steel plate costs $25. Stereo dissection microscopes ($1,200) were borrowed from general biology and plant taxonomy teaching laboratories. Because computers are becoming routine in teaching laboratories, programs for calculation of $V_m$ by the GHK equation are readily available (2, 9). The Membrane Potential Problem Solver is available for $95 (Labpack $220) with minimum equipment requirements of a 256K RAM, a CGA adapter, and a color monitor.

This exercise is remarkably consistent between classes (Table 1) and allows the testing of alternate hypotheses. Calculation of an expected $V_m$ using either the GHK or the NE equation provides a “target” value. Assessment of the quality of an impalement is essential in producing reproducible data. The first challenge for students is learning to make dependable microelectrode measurements. This may take up most of the initial laboratory time. However, once the technique is mastered, voltage measurements in higher K⁺ bathing solutions are acquired very quickly. Initial impalements in muscles bathed with 3.0 mM K⁺ usually produce voltages in the 50- to 50-mV range. Young experimenters are satisfied with these values until it is pointed that the GHK or NE equations predict a voltage between −70 and −90 mV. They then begin to check the quality of their microelectrodes and proceed to maneuver the pipettes more carefully. It becomes obvious from the expressions emitted by the groups of students when satisfactory high-voltage impalements are obtained. At the end of the laboratory period, voltage estimates from each $[K^+]_o$ are collected from the groups, pooled, and returned to the students so they may calculate average values and standard deviations. They then construct a graph similar to Fig. 1 and decide which algorithm best estimates the cellular response.

In my classes, I have chosen a series of $[K^+]_o$ that produces evenly spaced points on a semilog scale. As pointed out by Hodgkin and Horowitz (5), at $[K^+] < 10$ mM, the voltage prediction of the GHK and NE equations diverge. At higher $[K^+]_o$, the $V_m$ values predicted by the equations are indistinguishable. The current experimental protocol could be improved by impaling myofibers bathed with 1.0 mM K⁺ $V_m$ values collected under these conditions should produce $V_m$ values aligning with GHK rather than NE predictions. However, the inclusion of an additional concentration extends the experiment beyond a 3-h period (see Added proof).

Once students have processed the experimental data, they are then instructed to recount the assumptions of the two algorithms and explain why the GHK equation more accurately predicts $V_m$ than does the NE equation. The NE algorithm assumes a singular maximum permeability for K⁺, whereas the GHK assumes a K-to-Na permeability of 20:1. The Membrane Potential Problem Solver permits alteration of the K-to-Na ratio relative permeability and graphically illustrates concomittant changes in $V_m$. 
This asset helps students visualize the significance of permeability in the GHK equation. Although beyond the scope of the computer program, the students are asked to calculate a $V_m$ for a K-to-Na permeability ratio of 1:20 to illustrate the impact of the electromotive force of sodium. It should be recalled that the Ba$^{2+}$-mediated depolarization demonstrates the dependence of $V_m$ upon K$^+$ conductance as well as gradient. Later, this concept of relative permeability becomes helpful when discussing the basis of the action potential.

**Added proof.** Recently, this experiment was repeated in our teaching laboratory. After thirteen impalements, an average $V_m$ of $-84.1 \pm 3.0$ (SD) mV was obtained from muscle cells exposed to a Ringer solution containing 1.0 mM K$^+$. For [K]o of 1 mM, the GHK equation predicts a value of $-82.3$, whereas, for the NE equation, the value is $-121$. This $V_m$ measurement is indistinguishable from the GHK prediction line in Fig. 1. Inclusion of this concentration would help students assess which algorithm is best for predicting membrane voltage.

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**References**