Advances in Biology Laboratory Education
Publication of the Association for Biology Laboratory Education
Volume 42, Article 67, 2022

Temperature Dependence of the Passive Effects of K^+ on Membrane Potential of Skeletal Muscle as an Educational Module

Janki Naidugari[1], Dara Buendia Castillo[1,2], Mohammad Abou El-Ezz[1], Cameron Brown[1], Tom Calderaro[1], Cameron Evans[1], Trey Grant[1], Rachel Hazelett[1], Cassity High[1], Tessa Ilagan[1], Jack Klier[1], Nicole Marguerite[1], Felicia Marino[1], Shelby McCubbin[1], Nicholas Meredith[1], Blair Nethery[1], William Russell[1], Noah Sommers[1], Esther E. Dupont-Versteegden[2,3], Rebecca Krall[4], Katherine Sharp[4], Jeffrey Chalfant[4], Mia Brown[4], and Robin L. Cooper[1,2]

[1]675 Rose St, Department of Biology, University of Kentucky, Lexington KY 40506-0225 USA
[2]Center for Muscle Biology, 900 South Limestone, University of Kentucky, Lexington KY 40536-0200 USA
[3]Department of Physical Therapy, 900 South Limestone, University of Kentucky, Lexington KY 40536-0200 USA
[4]Rm 105 Taylor Education Bldg, 597 S. Upper St, Department of STEM Education, University of Kentucky, Lexington, KY 40406-0001, USA

(janki.naidugari@gmail.com, buendiad@uky.edu, mohammad.abouelezz@uky.edu, Cameron.E.Brown@uky.edu, tcalderaro15@uky.edu, Cameron.Evans@uky.edu, Trey.Grant@uky.edu, rachel.hazelett@uky.edu, Cassity.High@uky.edu, tessa.ilagan@uky.edu, jeklier12@uky.edu, Nicole.Marguerite@uky.edu, Feliciamarino@uky.edu, Shelby.McCubbin@uky.edu, meredithn@uky.edu, bvne222@outlook.com, William.Russell@uky.edu, Noah.Sommers@uky.edu, Esther.Dupont@uky.edu, rebecca.krall@uky.edu, katherinesharp@uky.edu, jeffrey.chalfant@uky.edu, mya.brown@uky.edu, RLCOOP1@uky.edu)

The effects of temperature on membrane potential are not commonly addressed in experimental physiology and neurophysiology student laboratory exercises even though temperature is often used in the treatment of medical conditions such as cardiac arrest, sepsis, and other maladies. This paper describes an authentic investigation students in a neurophysiology laboratory course embarked upon to explore the additional effects of temperature on the resting membrane potential in relation to the concentration of extracellular potassium ions $[K^+]_{o,}$. The laboratory investigation was modified from a typical membrane potential exercise using skeletal muscle of a crayfish as an

experimental model to obtain data because of the robustness of its preparation for student laboratories. The investigation explored three topics: (1) empirically study the effects of temperature and $[K^+]_o$ change on the Crayfish, (2) clinical therapies using changes in temperatures in mammals, and (3) the unified issue among organisms of temperature changes in cellular metabolism. Graphing membrane potential in relation to both $[K^+]_o$ and temperature along with theoretical curves for the Nernst and Goldman-Hodgkin-Katz (G-H-K) equations provided important insight into the relationship of temperature in these equations. Software freely available online was used in the calculating the theoretical values for changing the variables in the G-H-K equation. Discussion of other factors impacted by temperature for biological membranes were also discussed. A pre- and post-survey on general content and views on the activities was obtained.

Keywords: inquiry-based learning, membrane potential, temperature, equilibrium potential

Introduction

What is the purpose of undergraduate biology laboratory experiences, particularly in upper level coursework? The common laboratory exercise is designed around a traditional, "cookie cutter" lab where significant scaffolding characterized by step-by-step procedures results in a known outcome. Learners are then tasked with following the procedures to produce an outcome within an acceptable limit of error. What implicit message do these traditional laboratory experiences teach emerging biologists about the endeavor of science?

In his book, *What Is Life? How Chemistry Becomes Biology*, Addy Pross (2012) articulates the inductive nature of science. At its simplest form, he argues, "Ultimately all scientific explanations are inductive—they involve no more than the recognition of patterns and the association of the specific within the general. Broadly speaking the wider the generalization, i.e., the greater the number of empirical observations that are embraced by the generalization, the greater its predictive power and the more significant the generalization," (p. 46). How do biology students sharpen their inductive skills to discern patterns in the data and translate and then apply knowledge from the research literature to construct explanations or refute claims if they are only participating in traditional laboratory course exercises?

The investigation presented in this paper is designed to provide alternative tweaks to a standard protocol for novel investigative purposes and to engage students in learning the related content as they work as bench researchers in conducting a scientific study. We refer to this type of course-based investigation as an Authentic Undergraduate Research Experience (ACUREs) as these allow novel projects to be developed with the goal of publishing the findings; offering students a window into how science works and how results from their investigation add to the broader knowledge base of science (Auchincloss et al., 2014). In the past, this general resting membrane potential lab with varied extracellular concentration of potassium ions (i.e. $[K^+]_o$) was taught through the concept of muscle or neuronal injury and the spillage of K^+ that add to $[K^+]_o$. (Cooper et al., 2019). A twist was created in the current investigation using homogenized muscle (i.e., muscle juice) at various dilutions and examining the resulting membrane potential (Thenappan et al., 2019). An additional perturbation that was of particular interest to students was to explore the effects of muscle juice on evoked synaptic transmission and the occurrences of spontaneous quantal events as well as shapes in the evoked and single quantal events (Thenappan et al., 2019). The preparation offers unique experimental outcomes and potential follow up experimentation.

For example, since crayfish and many insects use glutamate as the neurotransmitter at the neuromuscular junction, the glutamate receptors become desensitized during the muscle juice application. This was likely due to the amount of glutamate within the homogenized muscle. The results led students to design new experiments investigating the effect of adding small amounts of glutamate to the $[K^+]_o$ in attempts to mimic the responses observed with the muscle homogenates. This past ACURE project was published presenting the novel results (Malloy et al., 2017). Thus, there are many approaches to developing projects where the students can contribute to novel research.

To assess student learning outcomes in conducting this module, we used a modified survey presented in Thenappan et al., (2019) to focus on the effects of temperature on the relationship with biological membranes and the effects of changes in ionic composition of intra- and extra-cellular fluids. In

addition, the survey included open responses to gather information on their views of the investigation.

Here, we present a detailed protocol along with student evaluations and empirical data from the experiment. Data will be provided on a web page as well as supplemental information for instructors to use in hybrid or remote teaching in case the hands-on experimentation in a laboratory is not possible. In virtual settings, instructors can provide data for students to graph and then discuss the findings.

Student Outline

Objectives

The students will learn:

1. How to take intracellular recordings from muscle fibers.
2. To apply the Nernst and Goldman-Hodgkin-Katz equation to derive theoretical membrane potentials.
3. How varying $[K^+]_o$ affects the membrane potential theoretically and experimentally.
3. How temperature affects the membrane potential theoretically and experimentally.
4. How to graph the theoretically and experimentally obtained data and make comparisons.
5. How to practically apply knowledge of the effects of varying $[K^+]_o$ and/or temperature on the physiology of cells, tissues and whole organisms.

Introduction

A common student physiology laboratory protocol for teaching content on the resting membrane potential is generally to vary the extracellular potassium ions $[K^+]_o$ in relation to the membrane potential. In experimentally determining this relationship, participants will vary the $[K^+]_o$ while taking measurements of the membrane potential with intracellular recordings. The experimentally obtained values are to be plotted in relation to the theoretical Nernst equation. The deviation in the curves will likely bring up the topic of the Goldman-Hodgkin-Katz (G-H-K) equation and why the G-H-K equation is a better fit for the experimentally obtained data (Atwood and Parnas, 1968; Baierlein et al., 2011; Johnson et al., 2014; Wyttenbach et al., 1999).

In this exercise, students will measure membrane potentials in a crayfish muscle and then vary $[K^+]_o$ at various temperatures to explore a possible effect of temperature on membrane potential. Specifically, the research question guiding this investigation is: How does variance in $[K^+]_o$ and temperature affect the membrane potential? Theoretical graphs will be constructed using a free online software program to show a relationship between membrane potential and variations of $[K^+]_o$, and a possible of effect of temperature on membrane potential with variations in $[K^+]_o$. The effect of temperature in relation to the curves will also to be calculated and discussed.

Relating this topic to real world applications, three general themes are presented for contemplation. The first theme is to address the topic with heterothermic animals, such as crustaceans and insects, that can be exposed to wide and abrupt variations in temperature rather than seasonal changes that occur gradually or the longer-term climate change. In addition, the effects on cells within a hibernating animal, such as a squirrel, also are discussed. The second theme addresses the potential implications of using temperature in clinical therapies, such as a cold or warm pack placed on injured tissue and a cold-induced coma used for reducing neural and heart function in cardiac and stroke patients. The third and final theme discussed in the commonalty regards the thermal changes that occur during cellular metabolism in heterotherms and homeotherms.

Methods and Data Collection

Exercises in Part A are to be conducted as pre-lab activities in preparation for anticipated observations in experiment.

Part A: Theoretical calculations and empirical measures of membrane potential

A Review of the Nernst equation and Goldman-Hodgkin-Katz (GHK) equation.

Equations that are commonly used to determine the equilibrium potential of an ion and the resting membrane potential are the Nernst equation and the Goldman-Hodgkin-Katz (G-H-K) equation, respectively. An important distinction between the two equations is that the Nernst equation is used only to determine the equilibrium potential for one specific ion, whereas the G-H-K equation is used to determine the resting potential by considering the

permeability of multiple ions and their gradients across a cell membrane (Nernst, 1888, 1889; Goldman, 1943; Hodgkin and Huxley, 1952; Hodgkin *et al.,* 1952; Hodgkin and Katz,1949; see Hille, 1992).

The Nernst equation is generally considered for ions across a membrane generating an electromotive force as commonly shown as:

$$V = \frac{RT}{zF} \cdot ln\frac{[X]_{out}}{[X]_{in}}$$

X = ion of interest
V = equilibrium voltage for the X ion across the membrane
R = gas constant [8.314 J/(mol•K)]
T = absolute temperature [Kelvin]
Z = valence of the ion
F = Faraday's constant [9.649 × 104 C/mol]

Calculating the K^+ ion at 20°C and transformation of ln to $\log_{10}$ is shown below using the following equation. After filling in the constants, one arrives at:

$$Potential = 58\ log\frac{[K]_{out}}{[K]_{in}}$$

Let us assume that only K^+ is permeant by diffusion. $[K]_{in}$ is the K^+ concentration on the inside of the cell and $[K]_{out}$ is the K^+ concentration on the outside of the cell.

As an exercise estimate $[K]_{in}$. _____

Assume for this calculation, membrane potential is only dependent on the K^+ equilibrium potential.

Given the $[K]_{out}$ = for the saline used is 5.4 mM. Also, assume membrane potential is -70mV.

$$Equilibrium\ Potential = 58\ log\ \frac{5.4}{[K]_{in}}$$

Solve for Potential:

-70/58=log 5.4/$[K]_{in}$

-1.2069= log 5.4/$[K]_{in}$

Antilog -1.2069= 5.4/$[K]_{in}$

$10^{-1.2069}$= 5.4/$[K]_{in}$

0.0621= 5.4/$[K]_{in}$

$[K]_{in}$ =5.4/0.0621=86.95 mM

Double check

X= 58 Log (5.4/86.95)= -69.999 so close enough to -70 mV

Considering that a membrane can be permeable to more than one ion at rest, as well as at various depolarized states, one uses the G-H-K equation to consider the permeability (P in the equation) for various ions. The G-H-K equation will reduce to the Nernst equation for a given ion if a membrane is permeable to only one ion.

Here is a generalized G-H-K equation for Na^+, K^+, and Cl^- ions:

$$Em_{K,Na,Cl} = \frac{RT}{F} ln \frac{P_{Na^+}[Na^+]_{out} + P_{K^+}[K^+]_{out} + PCl[Cl]in}{P_{Na^+}[Na^+]_{in} + P_{K^+}[K^+]_{in} + PCl[Cl]out}$$

Em = the collective equilibrium potential for the ions in the equation.

R = gas constant [8.314 J/(mol•K)]
T = absolute temperature [Kelvin]
F = Faraday's constant [9.649 × 104 C/mol]
P= permability of the ion

Since Cl^- has a negative charge, the concentration term is inverted in this equation for the inside and outside. This allows the Z (ion charge) to be left off.

A free software database allows one to put in different values for temperature and other variables in the G-H-K equation for a quick response in the expected membrane potential. The use of the rapid computer simulation demonstrates the effect of the various parameters on the membrane potential as well as how slight changes in one parameter can have a large role in the outcome.

The main emphasis for this exercise is to show how different temperature values and $[K^+]_o$ affect the membrane potential.

Use the given values reported in the literature for crayfish muscle as a reference. The concentration of K^+ in the saline to start off with is 5.3 mM and the temperature is 21^{o}C (Note: One needs to take the temperature of the saline in which the experiments are to be conducted).

There are potentials values estimated for crayfish neurons (Atwood 1982) reported in the literature that are included in the calculation along with those obtained from muscle fibers.

$[Na^+]_{in}$ = 17.4 mM (for neurons, Atwood 1982)

$[K^+]_{in}$ = 265 mM (for neurons, Atwood 1982)

$[Cl^-]_{in}$ = 12.7 (for neurons, Atwood 1982)

P_K = 1 (for neurons, Atwood 1982)

P_{Cl} = 0.1 (for neurons, Atwood 1982)

P_{Na} = 0.001 (for neurons, Atwood 1982)

For crayfish muscle:

"*Procambarus* and *Astacus* $[K^+]_{in}$ appears to be 171 and 167 mM, respectively" (Katz et al., 1972). One will use the values for *Procambarus* since this is the genus being used.

$[K^+]_{in}$ = 171 mM (determined for crayfish muscle)

$[K^+]_{out}$ = 5.3 mM (Saline)

$[Na^+]_{in}$ = 17.4 mM (assume for muscle)

$[Na^+]_{out}$ = 205 mM (Saline)

$[Cl^-]_{in}$ = 12.7 mM (assume for muscle)

$[Cl^-]_{out}$ = 232.15 mM (assume from saline; 205 mM NaCl; 5.3 mM KCl; 13.5 mM $CaCl_2 2H_2O$; 2.45 mM $MgCl_2\,6H_2O$)

P_K = 1 (assume for muscle)

P_{Cl} = 0.01 (assume for muscle)

P_{Na} = 0.001 (assume for muscle)

Use the on-line simulator from https://www.physiologyweb.com/calculators/ghk_equation_calculator.html

(Note: values of temperature are in K which is 273.15 + the centigrade temp)

Graph the results obtained from the online simulation for membrane potential based on the above values and for 5.3 mM, 10 mM, 20 mM, 30 mM, 40 mM, and 50 mM for $[K]_o$ and using 5ºC, 10ºC, 21ºC, and 30ºC. Plot in a similar manner to those obtained for the barnacle muscle (Figure 1; copied from Fischbarg, 1972; https://doi.org/10.1113/jphysiol.1972.sp009886).

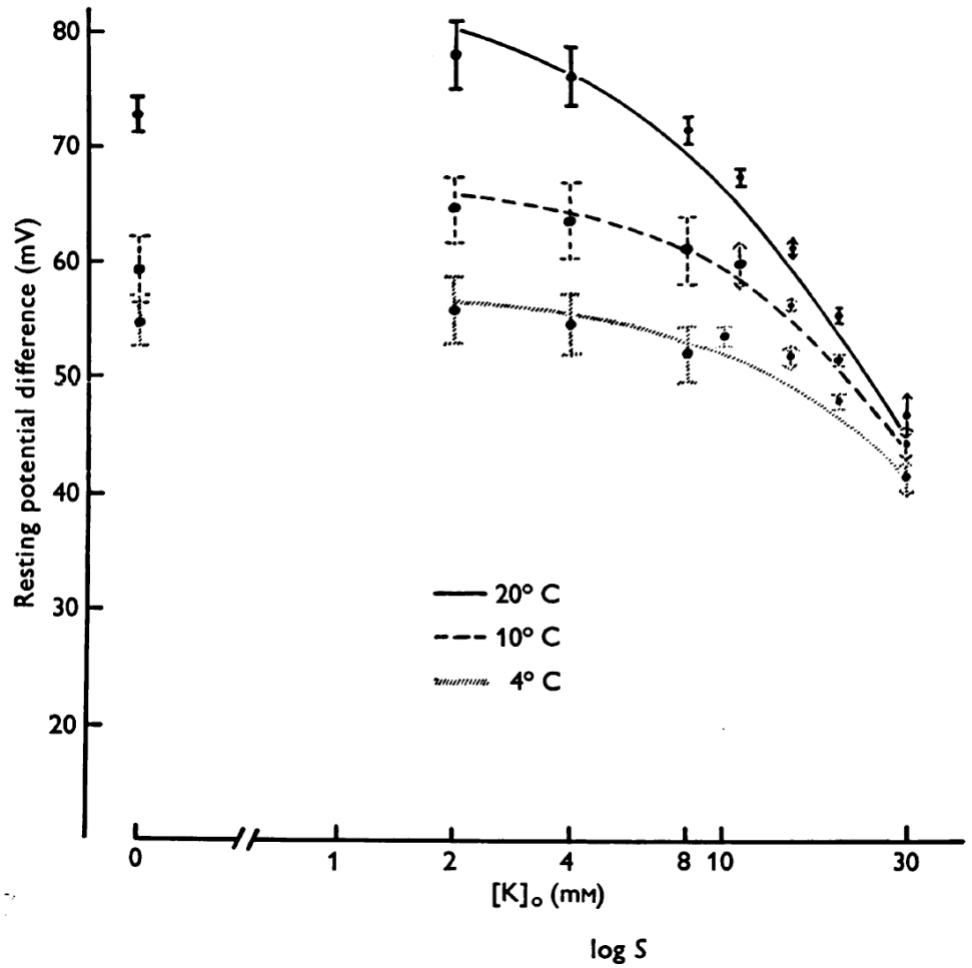


Figure 1: The effect of extracellular K^+ and temperature on the membrane potential in barnacle muscle. Copied with permission from Fischbarg (1972). Note the Y-axis is a change in the membrane potential becoming more negative with reduced extracellular K^+ and higher temperature. https://doi.org/10.1113/jphysiol.1972.sp009886

This concludes Part A exercises. Continue to Part B when in the laboratory.

Part B: Experimental measures

In this laboratory we are exploring the possible effect of various levels of $[K^+]_{out}$ and temperature on membrane potential. To collect experimental data, you will use sharp intracellular electrodes inserted into muscle fibers from a freshly dissected crayfish to measure membrane potential while changing the surrounding environment. Crayfish are used as the experimental organism as they survive for long periods of time with minimal saline composition. See Appendix A for details on dissection and electrophysiology set up.

Data Analysis

The $[K^+]_{out}$ and temperature values are varied, and the data is to be plotted and compared against the curves that were obtained from the computer simulations above. See Appendix A to fill in the data table and to make graphs.

Discussion and thought problems

As early as 1902, Bernstein was dealing with measuring resting membrane potential in the axon of a squid. It is intriguing to consider how these early ideas and observations of Berstein (1902) and Nernst (1888) later influenced research in membrane physiology. (See review by Malmivuo and Plonsey, 1995; also available on the www http://www.bem.fi/book/). There are still, to this day, breakthroughs being made about ion channel function and properties of biological membranes that are very significant in understanding the cellular physiology which relates to the function of tissues, organs and systems, as well as many pathologies. How do your findings add to our understanding of the effects of K+ and temperature on membrane potential?

The comparison of the experimental and theoretically derived effects of external $[K^+]$ on the resting membrane potential indicates the influence of ions on the membrane potential. Additional experiments using this same preparation remain to be performed to address fundamental physiological questions. Some were highlighted back in 1968 by Atwood and Parnas and have yet to be fully elucidated. With the techniques applied in this investigation, many questions can be explored in other experimental preparations as well as in physiological applications related to medicine and health. In the current investigation, we have demonstrated the usefulness of an invertebrate model preparation to address fundamental questions pertinent to all animals.

With the knowledge gained on the electrochemical gradients of ions in the above investigation, you can apply the results and knowledge gained from the research to now advance to the excitability of membranes by examining synaptic transmission at neuromuscular preparations in the crayfish. One can also apply these concepts on other cell types such as neurons and cardiac tissue.

Concepts to explore:

1. Ensure you can determine $[K^+]_{in}$ given all the other parameters in the Nernst equation.
2. Discuss why the estimated Ek may not match the resting membrane potential measured.
3. Why are the slopes of the lines for the graphs of membrane potential and $[K^+]_o$ not equal at different temperatures?
4. Why are the slopes not linear for the various $[K^+]_{out}$?
5. Explain why in the literature ratios of P_K/P_{Na} etc. are used rather than direct values when calculating membrane potential using the G-H-K equation?

Applications of K+ and Temperature Effects on Membrane Potential

1. Consider heterothermic animals, such as crustaceans and insects that can be exposed to wide variations in temperature. How might the temperature change effect the excitable cells such as muscle and neurons? Would lowering the body's temperature alter the threshold of a neuron or a heart cell? If so, would it be more or less excitable? Why or why not? Would the effect be just the opposite in excitability by the same amount if the temperature was raised (explain your reasoning)? Now consider a homeothermic animal such as a hibernating squirrel which can lower its body temperature with the environment. Would a similar effect occur in the cells of the squirrel with changing temperatures? How about the case for a human which functions best around a set temperature?

2. Hypothermia can be used as a type of therapeutic treatment. It is sometimes used for people who have a cardiac arrest, which happens when the heart suddenly stops beating. Once the heart starts beating again, healthcare providers use cooling devices to lower the body temperature for a short time to 89°F to 93°F (32°C to 34°C). The treatment usually lasts about 24 hours. Is this temperature change large enough to affect the resting membrane potential based on your experimental or theoretical/simulated calculations? Plug in the change to the online simulation and determine how much the membrane potential changed theoretically. What effect do you think it would have on membrane excitability?

   Consider the following copied from an article (Showman and Wedlick, 1963):

   > Conclusion
   >
   > In summary, we conclude that the local application of cold combined with proprioceptive neuro-muscular facilitation rehabilitation techniques is the most effective measure we have found in the treatment of multiple sclerosis. We have also found that, when mobilization of a joint is required, if there is any muscle

> spasm, then it seems preferable to precede reeducation techniques by the local application of cold rather than heat. The local application of cold would seem to be the ideal emergency measure in the initial treatment of acute muscle and ligament strains. It is our experience that, to be effective, cold therapy must consist of moist cold applied to the skin itself. The two methods available are either a spray technique or the use of ice and water. If the spray technique is used, then a fluoromethane spray, such as "Sketron", seems on all grounds far preferable to spraying with ethyl chloride; it has the advantage of being applied easily to any site. However, when muscle spasm is severe, we believe that the use of packs soaked in a mixture of ice and water seems to produce more lasting benefit than the local use of a cooling spray."

Consider your findings from the investigation, explain the physiological behind why these cold treatments work for muscle spasms? You might have seen on TV after major football games the athletes take ice baths. Do you think it is for the same reason to reduce muscle spasms? Are there any other physiological reasons for using ice baths in these cases?

3. Let us now switch over to metabolism of cells and heat production. When electrical activity of a neuron is induced, a rise of CO_2 from cellular metabolism and production of H^+ occurs. The rapid diffusion of CO_2 across bilipid membranes can influence both intracellular and extracellular pH (Gutknecht et al., 1977). Cellular metabolism would also raise the local temperature, as you likely know well with just a small amount of exercise. So, is the increase in heat more of a positive-feedback or a negative feedback on cellular excitability of neurons and muscle cells? Lastly, what might be the logic behind one's brain shutting off if one overheats or if it becomes too cold?

Cited References

Atwood HL. 1982. Membrane physiology of crustacean neurons. In Podesta RB, Ed. Membrane physiology of invertebrates; New York, Marcel Dekker, Inc. pp.341–407.

Atwood HL, Parnas I. 1968. Recording from the crayfish abdominal extensor muscle preparation with microelectrodes. In: Experiments in physiology and biochemistry (Kerkut GA, ed), pp 307-330. London: Academic.

Baierlein B, Thurow A L, Atwood HL, Cooper RL. 2011. Membrane potentials, synaptic responses, neuronal circuitry, neuromodulation and muscle histology using the crayfish: Student laboratory exercises. Journal of Visualized Experiments, 47, Retrieved from https://www.jove.com/Details.php?ID =, 2322

Bernstein J. 1902. Untersuchungen zur Termodynamik der bioelektrischen Ströme. Pflüger Arch ges Physiol. 9:521-562.

Fischbarg J. 1972. Ionic permeability changes as the basis of the thermal dependence of the resting potential in barnacle muscle fibres. The J Physiol 224(1):149–171.

Goldman DE. 1943. Potential, impedance, and rectification in membranes. J Gen Physiol 27:37-60.

Gutknecht J, Bisson MA, Tosteson FC. 1977. Diffusion of carbon dioxide through lipid bilayer membranes: effects of carbonic anhydrase, bicarbonate, and unstirred layers. J Gen Physiol. 69(6):779-794.

Hille B. 1992. Ionic Channels of Excitable Membranes, 2nd ed., Sinauer Assoc., Sunderland, Mass.

Hodgkin AL, Huxley AF. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 117:500-544.

Hodgkin AL, Huxley AF, Katz B. 1952. Measurement of current-voltage relations in the membrane of the giant axon of Loligo. J Physiol (Lond) 116:424-448.

Hodgkin AL, Katz B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. J Physiol (Lond) 108:37-77.

Johnson BR, Wyttenbach RA, Hoy RR. 2014. Cruataceans as model systems for teaching neuroscience: past, present, and future. In: Nervous systems and control of behavior, the natural history of the Crustacea. (Derby C, Thiel M, eds). New York, NY: Oxford University Press.

Katz GM, Reuben JP, MacBerman, Dunham PB. 1972. Potassium redistribution and water movement in crayfish muscle fibers. J Comp Physiol. 80:267–283.

Malloy C, Dayaram V, Martha S, Alvarez B, Chukwudolue I, Dabbain N, D.mahmood D, Goleva S, Hickey T, Ho A, King M, Kington P, Mattingly M, Potter S, Simpson L, Spence A, Uradu H, Van Doorn JL, Weineck K, Cooper RL. 2017. The effects of neighboring muscle injury on proprioception responses in crayfish and crab. J Exp Zoology 327(6):366–379.

Nernst WH. 1888. Zur Kinetik der Lösung befindlichen Körper: Theorie der Diffusion. Z Phys Chem 3:613-637.

Nernst WH. 1889. Die elektromotorische Wirksamkeit der Ionen. Z Phys Chem 4:129-181.

Malmivuo J, Plonsey R. 1995. Bioelectromagnetism- Principles and Applications of Bioelectric and Biomagnetic Fields. New York: Oxford University Press

Showman J, Wedlick LT. 1963. The use of cold instead of heat for the relief of muscle spasm. Med J Aust. 2:612-614.

Sohn J, Mykles DL, Cooper RL. 2000. The anatomical, physiological and biochemical characterization of muscles associated with the articulating membrane in the dorsal surface of the crayfish abdomen. J Experimental Zoology 287:353-377.

Wyttenbach RA, Johnson BR, Hoy RR. 1999. Crawdad. A CD-ROM Lab manual for neurophysiology. Sunderland, MA: Sinauer Associates.

Materials

Materials for 1 set up:

- Scissors (1)
- Forceps (1)
- Silver Wire for ground wire (1)
- Microscope (1)
- Electrode Probe (1)
- Petri Dish with Sylgard on the bottom (1)
- Saline Solution (1)
- Potassium Solutions: 5.4mM (normal saline), 10, 20, 40, 80, 100 mM
- Bleach (Small Amount, use for the tip of the silver wire to build Ag-Cl)
- Bulb Pipette (1), to remove and add solutions
- Syringe (1)
- Amplifier/Acquisition System (1)
- Faraday Cage (1)
- Desktop/Laptop (1)
- Dissection pins (4)
- Crayfish (1)

Notes for the Instructor

Throughout this exercise, the students are focused on being able to determine how the external concentrations of K^+ affect the membrane potential and how this varies with temperature. When using the G-H-K equation to determine the theoretical resting membrane potentials, the estimated values may differ from the values being physically measured. Routinely the normal membrane potential found by students was approximately 50mV. The students should not be discouraged if their values are not close to the predicted values. This can be due to several different factors in relation to temperature, such as small changes in permeability in the ion leak channels, the external environment of the membrane differing from our expectations, the effect on the pumps (i.e., Na-K ATP pump) and exchangers as well as alteration in pH which also can influence membrane potential. As mentioned in the Fischbarg (1972) study of muscles in barnacles, the author states the differences from theoretical values are likely due to alteration in permeability of the potassium leak channels. If the students were so inclined, they could try different values of permeability in the simulation at the higher temperatures to try to fit the theoretical values to the experimental measures.

By having the students run through the simulation prior to the experimentation, the understanding that temperature is critical in altering the membrane potential, students noted an emphasis to exchange the bathing solution twice or three times and record the measures within a few minutes. In addition, students realized spurious data points will make it hard to graph the data, so some students took care in obtaining an average membrane potential by recording two or three fibers quickly at each condition. The concern with using the simulation is having values to use for intracellular ion concentrations.

There are few student-friendly experimental tissues with reported values in the literature. Even in the well-studied crayfish skeletal muscles, different values are reported for internal K^+ and Na^+ concentrations. This can lead to a discussion on …This also brought up discussion points on different cell types if the ionic concentrations are similar. This then lead back to why some cells have varied resting membrane potentials and different equilibrium potentials for the ions compared to other cells. It was surprising to some students that researchers have not determined ionic concentrations in tissues of mammals. It was also surprising for some when discussing skeletal muscle for Drosophila that the equilibrium potential for Cl^- ions is a value more depolarized than the resting membrane potential (Rose et al., 2007). How the values of permeability of the ions are obtained was also a discussion point. Thus, an appreciation of past research and the shear amount of research required to determine such parameters began to be understood by the students. The realization that the squid axon was a fundamental preparation because the internal and external ionic concentrations could be exchanged to alter membrane potentials and calculate equilibrium potential was a point driven home in a instructor facilitated discussion.

As for the implementation of the experimental collection of data in a large class setting, it is hard to maintain temperature in the recording dishes. It would be useful to have a thermometer at each recording setup so a recording can be made while the membrane potential is recorded. The other issue in implementing this exercise is the breaking of the electrode tip in the recording dish. Sometimes the electrode tip may hit the cuticle or a dissection pin and the 3 mM KCl can leak out over the preparation before the bathing media can be exchanged with fresh saline.

It was observed that some students tried to keep the electrode in a muscle fiber while exchanging the bathing media. This may lead to inaccurate recordings due to alterations in the zero offset of the electrode and not testing if the tip of the electrode had been broken with the potential of the muscle twitching while exchanging the bath. It would be best to remove the electrode from the muscle fiber and re-zeroing the potential as well as testing the electrode resistance prior to taking the next recording. In addition, taking

an average of three recordings in different muscle fibers for each setting is beneficial for knowing if a data point is spurious.

Graphing the simulated and experimental data required some discussion for students to understand that there are various ways the concept is presented in the literature and textbooks. Some reports only indicate a difference in the membrane potential without the absolute values being reported. Also, some reports show a change as increasing on the y-axis with hyperpolarization, which can be confusing. It may be useful to indicate reporting values on the y-axis as the absolute membrane potential as negative values indicated in a downward and depolarization in increasing in an upward direction. In addition, published reports sometimes indicate a ratio of $[K]_{out}/ [K]_{in}$ as compared to only $[K]_{out}$.

In discussing practical applications of this laboratory exercise, a theme of using poikilotherms and environmental changes in temperature effects on the membrane potential is readily understood. A discussion in ionic and osmolarity changes with seasonal changes in temperature for insects is an interesting topic for more discussion and searching the primary literature for students in writing their lab reports. Moving the discussion from poikilotherms to homeotherms which drop their body temperature along with environmental temperature draws attention to this exercise. One idea to discuss is measuring membrane potentials in cells of hibernators and non-hibernators to see if there are differences in adaptation among species at different temperatures.

Many life science undergraduate students are interested in health care as a future career. To pique an interest in these students a discussion in the use of therapeutic hypothermia used in humans to recover from heart attacks, strokes, brain damage or after brain surgery to generally prevent the brain from further damage or insult was approached. Therapeutic hypothermia is used to reduce metabolism and swelling of neural tissue until some time has passed to reduce the initial trauma. However, physicians do not want to reduce body temperature too much as the heart can become arrhythmic and can even stop beating as well as clotting issues with blood. The mechanism of why the heart stops beating when exposed to cold has yet to be determined. However, the heart tube in larval have been rejuvenated when Ca^{2+} ions are allowed to flux across the cardiac cells in larval *Drosophila* placed in the cold for long periods. Such studies in larval *Drosophila* were conducted with optogenetic techniques (Zhu et al., 2016 a, b), which demonstrated that the mechanical ability of the heart to beat is possible in the cold but only when the light sensitive ionic channels where activated.

Cited References

Cooper RL, Thenappan A, Dupont-Versteegden EE. 2019. Examining motor and sensory units as an educational model for understanding the impact of localized tissue injury on healthy cells. Article 6. In: McMahon K, editor. Tested studies for laboratory teaching. Volume 40. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). Vol. 40, Article 6, 2019

Fischbarg J. 1972. Ionic permeability changes as the basis of the thermal dependence of the resting potential in barnacle muscle fibres. The J Physiol. 224(1):149–171.

Malloy C, Dayaram V, Martha S, Alvarez B, Chukwudolue I, Dabbain N, D.mahmood D, Goleva S, Hickey T, Ho A, King M, Kington P, Mattingly M, Potter S, Simpson L, Spence A, Uradu H, Van Doorn JL, Weineck K, Cooper RL. 2017. The effects of neighboring muscle injury on proprioception responses in crayfish and crab. J Exp Zoology 327(6):366–379.

Pross A. 2012. What is Life?: How Chemistry Becomes Biology. Oxford Landmark Science. OUP Oxford, ISBN 0191650897, 9780191650895.

Rose U, Derst C, Wanischeck M, Marinc C, Walther C. 2007. Properties and possible function of a hyperpolarisation-activated chloride current in Drosophila. J Exp Biol. 210(14): 2489-2500.

Thenappan A, Dupont-Versteegden EE, Cooper RL. 2019. An educational model for understanding acute deep tissue injury of motor units. Journal of Young Investigators 36(5):62-71.

Zhu Y-C, Yocom E, Sifers J, Uradu H, Cooper RL. 2016a. Modulatory effects on Drosophila larva hearts in room temperature, acute and chronic cold stress. J Comp Physiol – B. 186 (7):829-841.

Zhu Y-C, Uradu H, Majeed ZR, Cooper RL. 2016b. Optogenetic stimulation of heart rate at different temperatures and Ca^{2+} concentrations. Physiol Reports. 4(3):e12695.

Acknowledgments

Thank you very much to all the Bio446 students and teaching assistants who have helped improve this laboratory exercise over the last four years.

About the Authors

Janki Naidugari, Mohammad Abou El-Ezz, Cameron Brown, Tom Calderaro, Cameron Evans, Trey Grant, Rachel Hazelett, Cassity High, Tessa Ilagan, Jack Klier, Nicole Marguerite, Felicia Marino, Nicholas Meredith, Blair Nethery, William Russell, and Noah Sommers were students in either Bio446 or Bio650 course (Neurophysiology Lab) who contributed to the data presented, survey data and to editing of this manuscript. Ms. Naidugari has taken the lead of presenting this project at the ABLE meeting and overseeing this project for the class presentations.

Ms. Shelby McCubbin is an undergraduate student at the University of Kentucky majoring in Neuroscience. She is continuing this experimental module as a research project.

Ms**.** Dara Buendia Castillo was the teaching assistant for the Bio446 course and helped to gather some preliminary data for these experiments.

Dr. Esther E. Dupont-Versteegden graduated from State University Limburg at Maastricht, the Netherlands, with a BS in Movement Sciences. In 1995 she received a PhD in Physiology from the University of Texas Health Science Center at San Antonio, Texas. In 2006 she accepted her current position at the University of Kentucky, College of Health Sciences.

Rebecca Krall currently is an instructor for science methods for preservice teachers and effective uses of technology for graduate students in STEM at the University of Kentucky. Her current research interests include developing K-8 teachers' scientific knowledge and pedagogical skills for creating authentic science experiences for students and the effect on student learning; and teachers' abilities to notice, interpret, and apply student thinking during their instruction. She earned a BA in education from Virginia Tech in 1988, a MEd and PhD in STEM Education from the University of Virginia in 2000 and 2004, respectively.

Katherine Sharp is an Assistant Professor of Chemistry at Stephens College. She earned a B.S. in Biology from Stephens College in 2015 and an M.S. in Chemistry and Biochemistry from the University of Oklahoma in 2018, and a PhD in Education Sciences (STEM Education emphasis) from the University of Kentucky in 2021. Her current research interests include creating authentic learning experiences in undergraduate chemistry classrooms and exploring how these experiences impact the development of critical thinking skills.

Jeffrey Chalfant is a Ph.D. student in the Department of STEM Education at the University of Kentucky. He received a M.S. in Biology from the University of Kentucky in 2020 where he conducted research on the effects of circadian disruption on cardiovascular disease. He now studies how students construct and ask scientific questions.

Mia Brown is a PhD student in STEM Education at the University of Kentucky. Her dissertation focus is on Critical Race Theory and examines factors contributing to personal and professional identity development and how intersecting identities impact interest, persistence, experience, and success in STEM of women of color. She earned her M.S. in Biological Sciences and M.S. in Adult and Technical Education from Marshall University. She is a Program Evaluation Research Associate in the Office of Health Affairs at West Virginia University.

Robin Cooper is an instructor of animal physiology and neurophysiology at the University of Kentucky. He received a double major with a B.S. from Texas Tech in 1983 and a PhD in Physiology from Texas Tech Medical School in 1989. He has been at the University of Kentucky since 1996.

Appendix A

Preparation/Dissection

1. A crayfish approximately 6-10 cm in body length should be obtained. Hold the crayfish at the back of the head or approximately a centimeter from the back of the eyes. Ensure that neither the claws of the crayfish nor its mouth can reach the individual handling the crayfish. (The crayfish may be placed in crushed ice for 5 minutes to anesthetize it prior to removing the head).

2. Once anesthetized, remove the crayfish from the ice and use the large scissors to quickly remove the head. Make a clean and quick cut from behind the eyes of the crayfish. Dispose of the head and appendages.

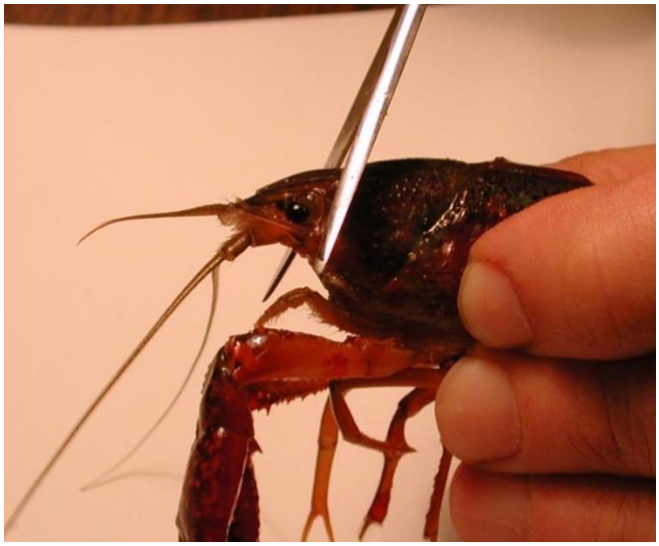


Figure 2: Image shows placement of the cut to remove the head of the crayfish.

3. The legs and claws of the crayfish can be removed at this point to avoid injury. Stylets on males and swimmerets on both males and females can also be removed (Figure 3). Next, separate the abdomen from the thorax. Make a cut along the articulating membrane, which joins the abdomen and thorax (Figures 4 and 5). Save the abdomen portion of the crayfish and dispose of the thorax.

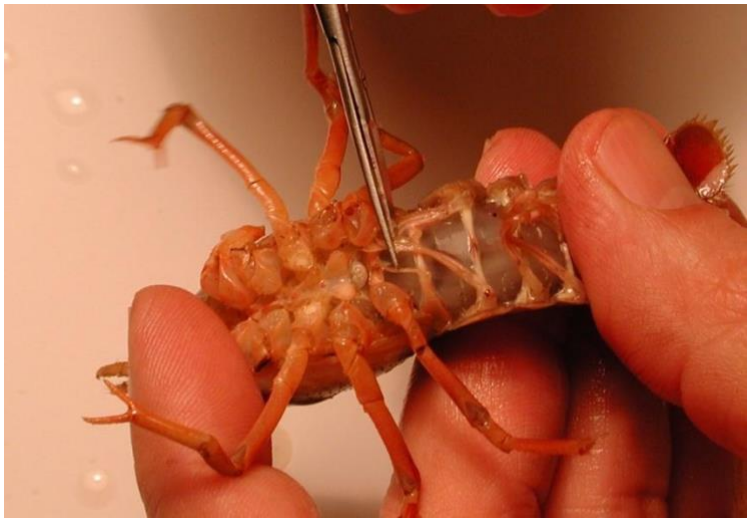


Figure 3: The scissors are cutting the stylets. These can be removed from the crayfish.

Figure 4: Image shows the placement of the cut to remove the thorax from the abdomen.

Figure 5: Removal of the thorax from the abdomen. The cut should be made in circular fashion along the line in the joining of the segments.

Figure 6: The top image shows the abdomen with swimmeret appendages. Bottom image shows the abdomen without the swimmeret appendages.

4. With the abdomen, a cut should be made in the shell along the lower, lateral border of each side of the abdomen. Care should be taken not to cut too deeply into the crayfish. To help in the process of cutting the shell, the cut should be made with the scissors pointing slighting down towards the ventral side and at an angle. Follow the natural shell pattern of lines of the crayfish that run the length of each segment (Figure 7).

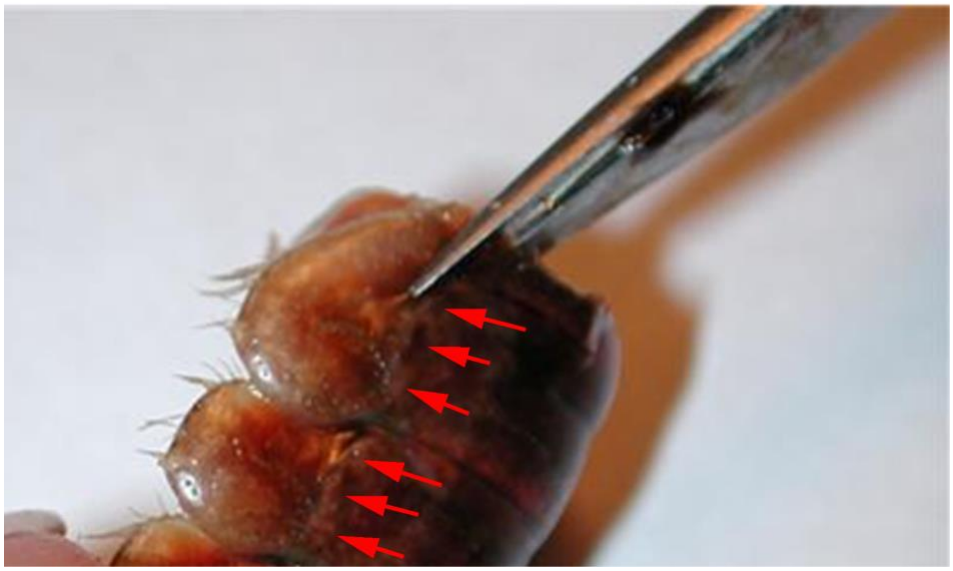


Figure 7: Scissors are placed at an angle and follow the natural alignment of the shell. Do not cut too deep and destroy the preparation. The arrowheads point to the natural line along each segment that should be followed for the cuts.

5. Remove the ventral portion of the shell. Take care not to destroy the abdominal muscles. Use forceps to remove the ventral portion. When the ventral portion of the shell is removed, a white mass of tissue can be seen on top of the deep flexor muscles. This tissue can be removed carefully with forceps.

Figure 8: Removing the ventral portion of the shell with forceps. Pull up and back on the ventral portion to remove. Do not destroy muscles under the ventral shell.

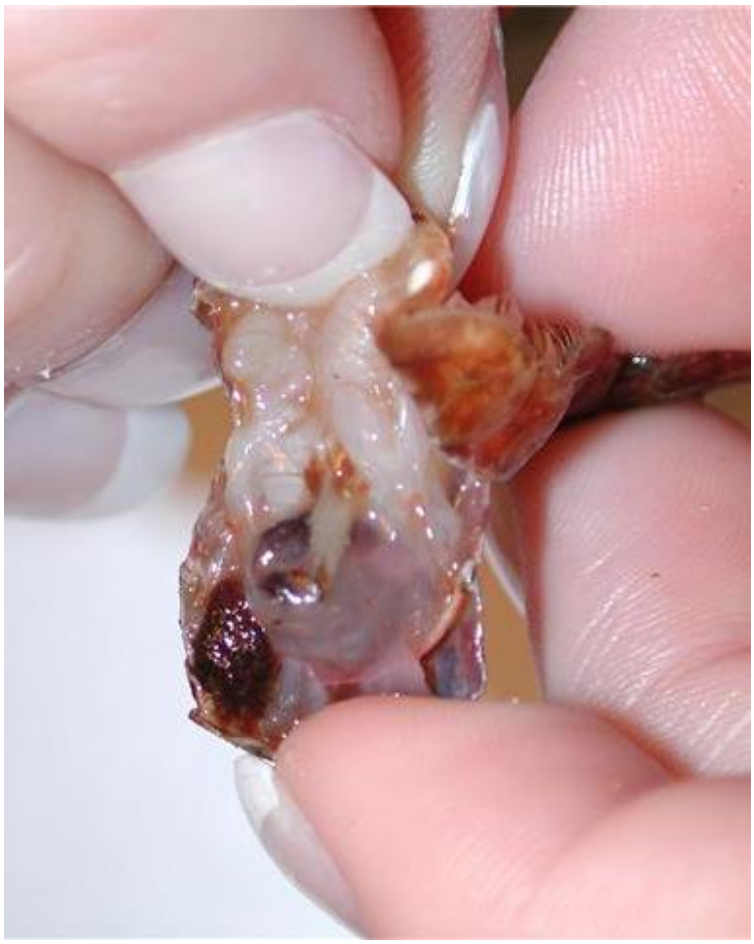


Figure 9: Pulling back on the ventral portion of the shell, which is to be discarded.

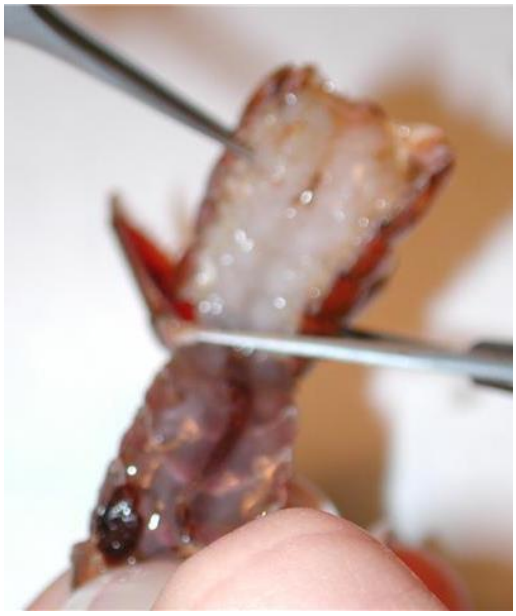


Figure 10: Cut the ventral portion of the preparation with scissors and discard.

6. The GI tract (Figure 11), a small tube running along the midline of the deep flexor muscles, can be removed from the crayfish. Pinch the top of the tract with the forceps and pull away from the abdomen. Cut the bottom of the tract – at the end of the tail. Rinse the dissection with saline to ensure the fecal waste does not interfere with the preparation.

Figure 11: Image shows the removal of the GI tract from the preparation.

7. Use dissection pins to secure the preparation to the Petri dish. The top and bottom corners of the preparation should be pinned down to the dish. To help with inserting the glass electrode into the muscle cells, slightly angle the preparation on its side and pin down. This will make movements with the micromanipulator easier (see figure 13). Saline solution should be poured into the Petri dish and cover the preparation completely until intracellular recordings are performed.

This dissection dish should have a Sylgard (Dow Corning) coating on the bottom (1cm thick) so that insect pins can be stuck into it.

Dissected preparations are bathed in standard crayfish saline, modified from Van Harreveld's solution (1936), which is made with 205 NaCl; 5.3KCl; 13.5 $CaCl_2$; $2H_2O$; 2.45 $MgCl_2$; $6H_2O$; 5 HEPES and adjusted to pH 7.4 (in mM).

Intracellular Recording

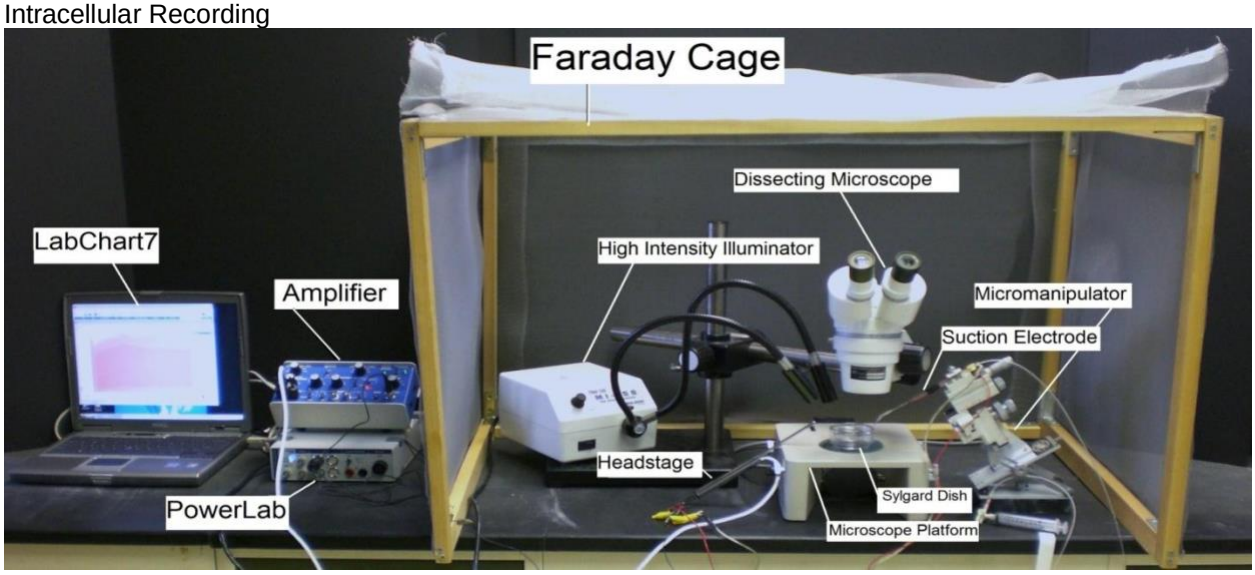


Figure 12: General setup of the recording equipment from. Specific set-ups may vary with different electrodes.

8. The specimen dish with preparation should be placed under the microscope and secured with poster tack beneath the dish to prevent movement.

Figure 13: Placement of the preparation under the microscope. Use poster tack (shown in blue) to secure the specimen dish and ground wire to the stage of the dissecting scope.

9. Two wires each with a short length of silver wire attached to one end should be obtained. The silver wire should be dipped into a small amount of bleach for about 20 minutes to obtain an Ag-Cl coating. Wash the wire with distilled water before using. A glass intracellular pipette should be obtained and carefully backfilled with a long needle attached to a syringe filled with a 3M KCl solution. The pipette should be turned down (sharp end pointing towards the ceiling) and filled with solution. This will ensure that any excess KCl will drip out the back of the electrode. Be sure no KCl runs along the glass pipette that will enter the saline bath. If KCl does spill over to the tip toss that one out and fill a new electrode. Turn the pipette upright when finished filling with potassium chloride solution. The silver wire can then be placed into the pipette (take care not to twist the pipette as it can break inside of the microelectrode). Intracellular recordings of resting membrane potentials used sharp glass electrodes (catalogue # 30-31-0 from FHC, Brunswick, ME, 04011, USA) filled with KCl (3 M) to obtain a 20-40 MΩ resistance were used. An agar bridge (1.5% agar in normal crayfish saline) was used to keep the electrical potentials from fluctuating when the saline was exchanged. The agar bridge was made with standard plastic Eppendorf pipette tips (200 µl) by using the small tip opening placed in the saline and the ground wire placed into the agar. A standard intracellular amplifier (A-M Systems, model 3000) was used which was bridged to a computer with an analog digital board (Power lab, model 26T, ADInstruments, Colorado Springs, CO). The signals were recorded and analyzed via Scope and LabChart software (ADInstruments, Colorado Springs, CO).

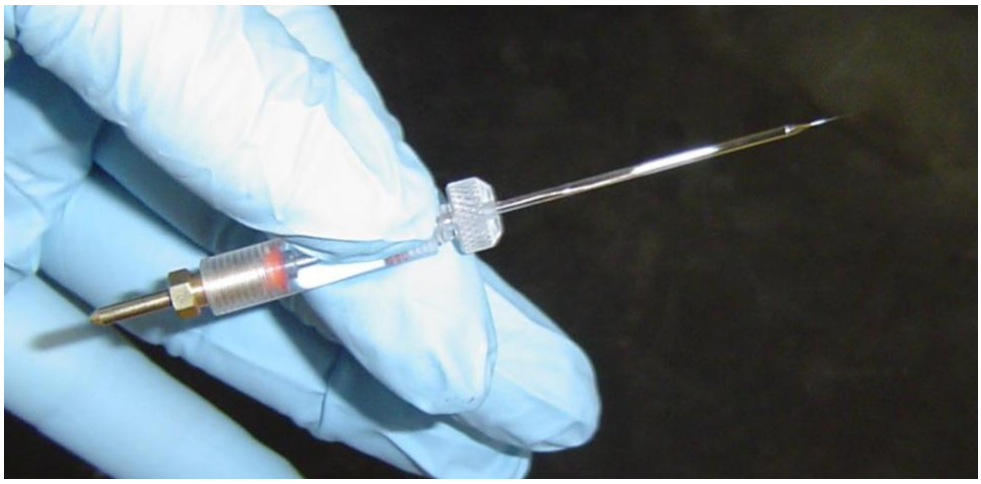


Figure 14: Microelectrode assembly with glass capillary in place.

The intracellular amplifier used during the intracellular membrane potential recordings has a button to use to test electrode resistance and can be read directly off the digital screen. The amplifier can be set-up to record 1 X or 10 X output on a computer based on which BNC output is used.

Software Set-up

10. Be sure your amplifier and PowerLab units are on before opening the software!
11. Open the LabChart software. Adjust the chart to display only one channel by clicking “Setup”, then “Channel settings.” Under “Channel settings,” change number of channels to one. Click “OK.”
12. At the top of the chart, left hand corner, cycles per second should be 2K. Set volts (y-axis) to around 1V.
13. Click on “Channel 1” on the right-hand portion of the screen. Click “Input Amplifier” and that the following settings are selected:

Single ended	OFF
Differential	Checked
AC-Coupled	OFF

Anti-alias	Checked
Invert	OFF

14. CHECK THE RESISTANCE OF YOUR ELECTRODE.

To measure the resistance, place the tip of the glass electrode into the saline bath. Make sure the ground wire is also in the saline bath. While recording, the Ω TEST switch should be turned on and then off several times. The amplitude (mV) of the resulting changes should be measured. To measure the amplitude changes in the trace, place the marker on the steady base line and then move the cursor to the peak amplitude off computer traces or read the values directly off the digital output on the amplifiers.

Average Resistance (MΩ) = _____

The resistance should be within 20 to 60 MegaOhms. Low (<20) and high resistance (>100) are not acceptable. Troubleshoot as necessary to bring your electrode's resistance within the acceptable range.

15. NEXT, SET YOUR EXTRACELLULAR VOLTAGE TO ZERO. Set the gain in your software to 1 or 5 V/div. Begin recording by pressing "start" at the bottom of the screen. Use the DC offset knob on the amplifier to adjust the recording trace to zero before inserting the electrode into the tissue. This sets your extracellular voltage to zero.

16. Use the micromanipulator and dissecting scope to insert the microelectrode tip into the longitudinal muscles (DEM or DEL1 or DEL2) of the preparation (see Figure 15 and 16). The electrode should barely be inserted into the muscle. You will likely see the muscle dimple as the electrode penetrates. Once you observe the muscle dimpling, you can lightly tap on the back of the micromanipulator to help penetrate the muscle cell. Do not penetrate completely ***through*** the muscle. This can be avoided by using the fine adjustment knob on the micromanipulator. The high intensity illuminator should be adjusted to clearly see the muscle as the electrode is being inserted. Try to limit light exposure when not manipulating the preparation because the heat from the lights can damage the preparation. When poking muscle fibers in this preparation one can commonly run into spaces and clefts within the muscle. This is the reason why the membrane potential can appear, then disappear, and then reappear.

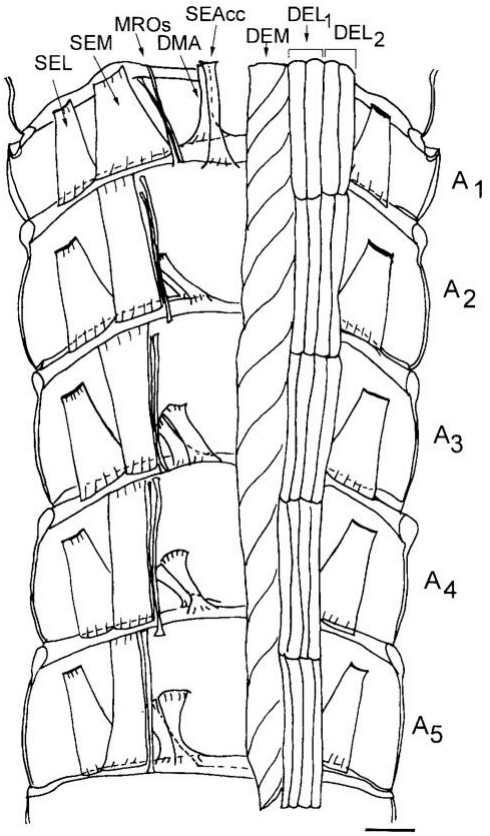


Figure 15. Schematic drawing from a ventral view of the dorsal part of the crayfish abdomen showing the extensor musculature of each segment. The dorsal membrane abdomen muscle (DMA) and the superficial extensor accessory muscle head (SEAcc) occur in segments 1 through 5 of the abdomen with a different orientation for each segment. With the exception of segment 1, these muscles have their attachment sites at their anterior end to the calcified tergite and at the posterior end in the articular membrane. In segment 1, the homologous muscles have their anterior attachment sites to the articular membrane located between the thorax and abdomen. The illustration was based upon photographic montages of methylene blue stained preparations. On the left side of the figure all the deep extensor muscles have been removed to show the dorsal superficial extensor muscles. Scale = 2.35 mm. (Taken from Sohn et al. 2000).

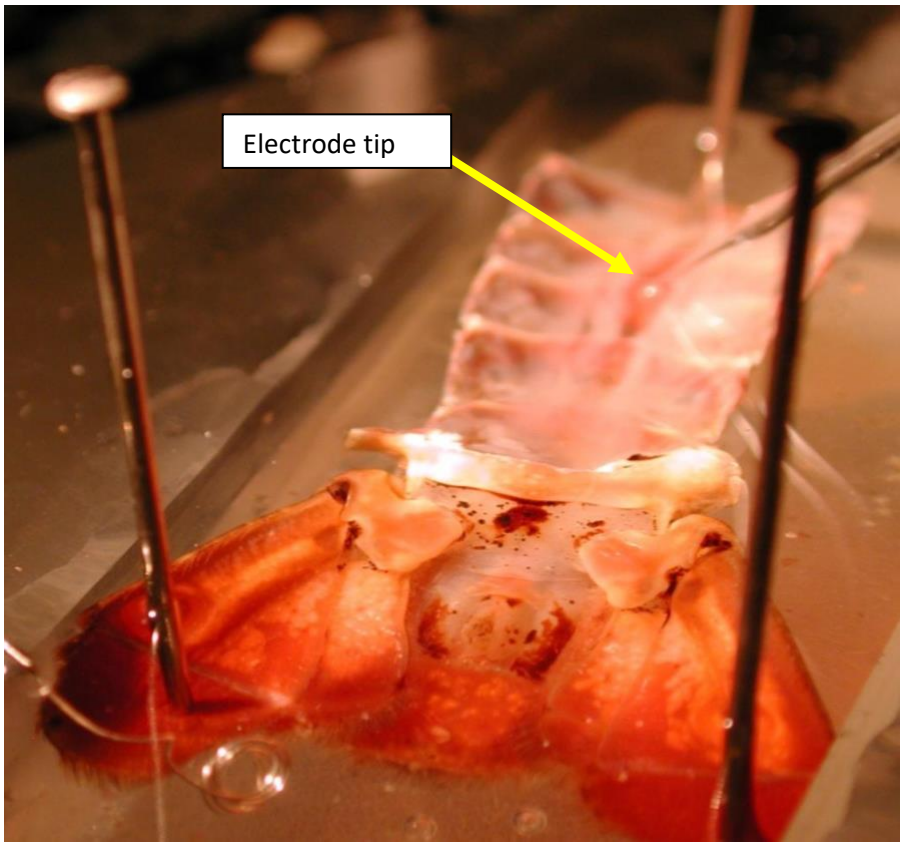


Figure 16: Insertion of electrode into the muscle of a pinned preparation to the recording dish.

The difference in the computer recorded values might need to be adjusted to account for any amplification used on the amplifier (i.e., 10X amplification). The voltage should be converted to millivolts if the values are reported on the software as volts (1 V = 1,000mV).

17. Carefully use the dissecting scope and micromanipulator to withdraw the electrode from the muscle. Reposition the electrode and insert the tip into another muscle fiber (this can be right next to your previous insertion). Record the resting membrane potential. One should collect several recordings and be comfortable with measures as well as directing the intercellular electrode into the muscle fiber of interest before moving onto the next step

18. The bathing solution can now be exchanged starting with 5.3 mM K^+ to the series of 10 mM, 20 mM, 30 mM, 40 mM, 50 mM K+ concentrations and then returned to 5.3 mM.

19. After this series of [K^+] was used then a second and third series was used but at 10^{o}C then 5^{o}C and finally at 30^{o}C. Use the higher temperatures last as they may damage the muscle fiber. A period of 5 minutes was used for initial conditioning to the new temperature before proceeding with the next series of [K^+] changes.

20. Fill in the table:

$[K^+]_{out}$ (mM)	Electrode resistance	Membrane potential (mV)	Bathing temperature
5.3			21
10			21
20			21
30			21
40			21
50			21
5.3			10
10			10
20			10
30			10
40			10
50			10
5.3			5
10			5
20			5
30			5
40			5
50			5
5.3			30
10			30
20			30
30			30
40			30
50			30

Student assessment

Preliminary results indicate significant learning occurs. Student participants (n=17, matched), in an Animal Physiology course (BIO350) at the University of Kentucky, were asked to complete a pre- and post-survey assessing students' learning outcomes related to the resting membrane potential (RMP). When asked about RMP and ion distribution, participants demonstrated a significant increase ($p=0.002$) in content understanding. Additionally, when asked about describing and performing calculations of RMPs, participants showed a significant increase ($p<0.001$) in their confidence levels. These data suggest that participation in a resting membrane laboratory exercise increases student content understanding and confidence about RMPs.

General Assessment Questions

The following questions were used as a pre-survey:

1. What ions are higher in concentration inside the skeletal muscle cytoplasm, when at a resting state, as compared to the extracellular fluid around a skeletal muscle?
A. Ca2+
B. Na+
C. K+
D. Cl-
E. I am not sure.

2. Invertebrates (such as a crayfish) can serve as a model to address some physiological phenomena in relation to mammals?
A. True
B. False

3. Which description below best describes what happens to the membrane potential when K+ ions increase (for example from 5 mM to 50 mM) in the extracellular fluid around a mammalian skeletal muscle?
A. The resting membrane potential of the muscle will not change.
B. The resting membrane potential of the muscle will hyperpolarize (become more negative).
C. The resting membrane potential of the muscle will depolarize (become more positive).
D. I am not sure.

4. Have you used the Nernst equation or the Goldman-Hodgkin-Katz equation to help in understanding the membrane potential of cells prior to this course?
A. Yes
B. No

5. When and invertebrate like a crayfish or an insect goes from a warm environment to a cold environment or the temperature around them changes from warm to cold what do you think will happen to the resting membrane potential of the muscle fibers
A. depolarizes
B. Hyperpolarize
C. does not change

Please explain your answer for question 5.

6. (Short answer)
Therapeutic hypothermia is a type of treatment. It's sometimes used for people who have a cardiac arrest. Cardiac arrest happens when the heart suddenly stops beating. Once the heart starts beating again, healthcare providers

use cooling devices to lower your body temperature for a short time. It's lowered to around 89°F to 93°F (32°C to 34°C). The treatment usually lasts about 24 hours.
During cardiac arrest, blood doesn't flow to the organs of the body. The brain may also not get enough blood. That's why many people don't recover after cardiac arrest. The lack of blood flow can cause lasting damage to the brain. The person may be unable to regain consciousness. Lowering the body temperature right away after cardiac arrest can reduce damage to the brain. That raises the chances that the person will recover.
Therapeutic hypothermia can help only some people who have had cardiac arrest. Therapeutic hypothermia can be a good choice if the heart restarted but if one is still not responsive. It can raise the chance that you will wake up.

How might lowering the body's temperature reduces brain damage and improve survival in your view?

The post-survey after completing the laboratory exercise

General assessment questions (mark A, B, C, D or E after each point)

Part 1. On a scale of 1 – 5: (A) Strongly agree, (B) Agree, (C) Neutral, (D) Disagree, (E) Strongly disagree:

1. This exercise increased my interests in knowing more about how tissue injury can affect whole body health in humans.
2. This exercise increased my understanding of the scientific method.
3. This exercise helped me to understand how the membrane potential is altered by temperature in muscle and neurons.
4. I have a better understanding of the physiological conditions that heterothermic (cold blooded) animals might have to deal with in their environment related to temperature changes.
5. After conducting this exercise, I feel comfortable developing my own hypotheses to address questions related to physiological functions of resting membrane potential of cells
6. After conducting this exercise, I feel confident that I can develop an experiment to test a hypothesis in a quantitative manner
7. After conducting this exercise, I feel confident conducting basic data analysis and summarization
8. I understand that ionic compounds inside cells that spill out during injury can be related to injury in other cells.
9. I feel comfortable developing a muscle fiber electrophysiological recording as part of an experimental study.
10. I feel more confident in my note taking abilities, and my ability to summarize general scientific trends

Part 2 (same questions as the 1st assessment but seeing if one has a different understanding now after conducting a laboratory exercise on this topic)

1. What ions are higher in concentration inside the skeletal muscle cytoplasm, when at a resting state, as compared to the extracellular fluid around a skeletal muscle?
A. Ca2+
B. Na+
C. K+
D. Cl-
E. I am not sure.

2. Invertebrates (such as a crayfish) can serve as a model to address some physiological phenomena in relation to mammals?
A. True
B. False

3. Which description below best describes what happens to the membrane potential when K+ ions increase (for example from 5 mM to 50 mM) in the extracellular fluid around a mammalian skeletal muscle?
A. The resting membrane potential of the muscle will not change.
B. The resting membrane potential of the muscle will hyperpolarize (become more negative).
C. The resting membrane potential of the muscle will depolarize (become more positive).

D. I am not sure.

4. Have you used the Nernst equation or the Goldman-Hodgkin-Katz equation to help in understanding the membrane potential of cells prior to this course?
A. Yes
B. No

5. When and invertebrate like a crayfish or an insect goes from a warm environment to a cold environment or the temperature around them changes from warm to cold what do you think will happen to the resting membrane potential of the muscle fibers
A. depolarizes
B. Hyperpolarize
C. does not change

Please explain your answer for question 5.

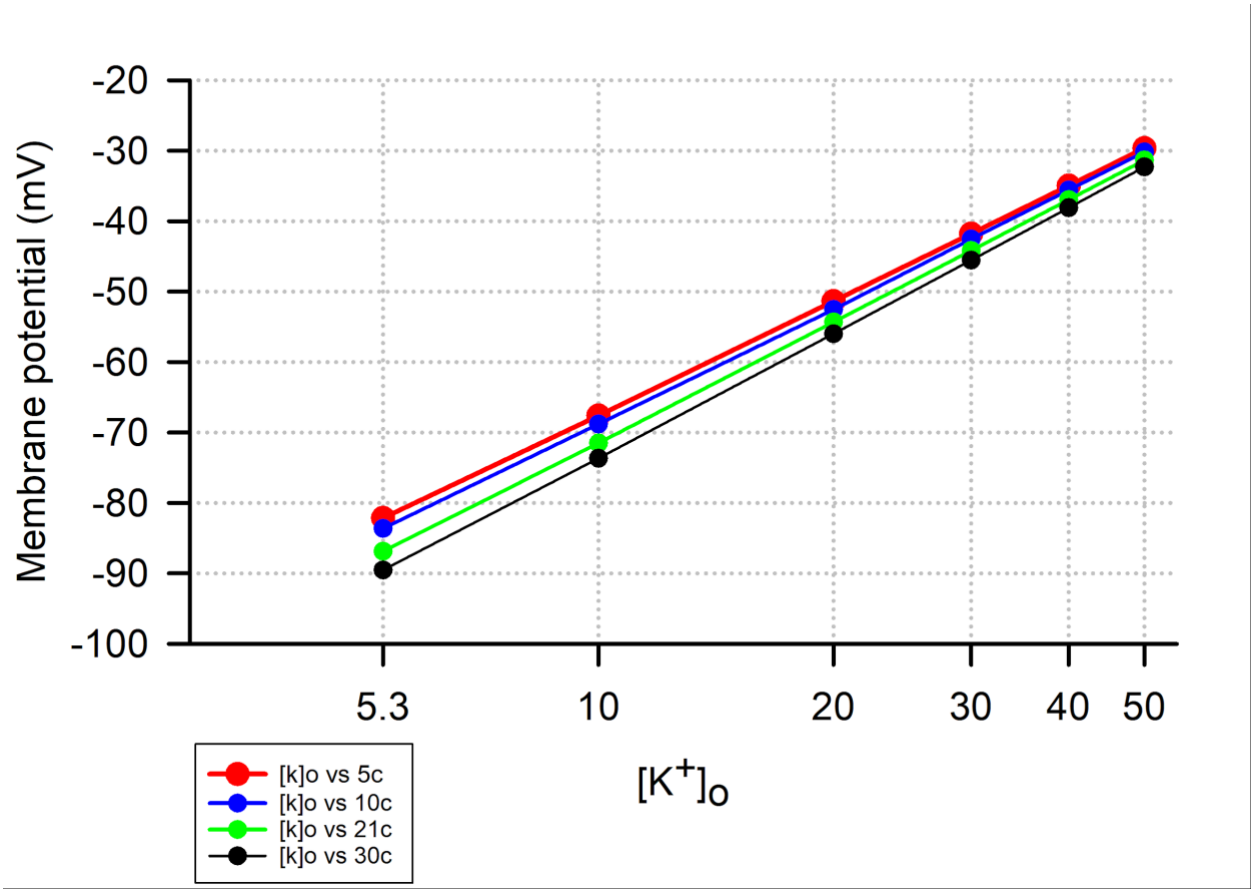
6. (Short answer)
Therapeutic hypothermia is a type of treatment. It's sometimes used for people who have a cardiac arrest. Cardiac arrest happens when the heart suddenly stops beating. Once the heart starts beating again, healthcare providers use cooling devices to lower your body temperature for a short time. It's lowered to around 89°F to 93°F (32°C to 34°C). The treatment usually lasts about 24 hours.
During cardiac arrest, blood doesn't flow to the organs of the body. The brain may also not get enough blood. That's why many people don't recover after cardiac arrest. The lack of blood flow can cause lasting damage to the brain. The person may be unable to regain consciousness. Lowering the body temperature right away after cardiac arrest can reduce damage to the brain. That raises the chances that the person will recover.
Therapeutic hypothermia can help only some people who have had cardiac arrest. Therapeutic hypothermia can be a good choice if the heart restarted but if one is still not responsive. It can raise the chance that you will wake up.

How might lowering the body's temperature reduces brain damage and improve survival in your view?

Sample results obtained by the students

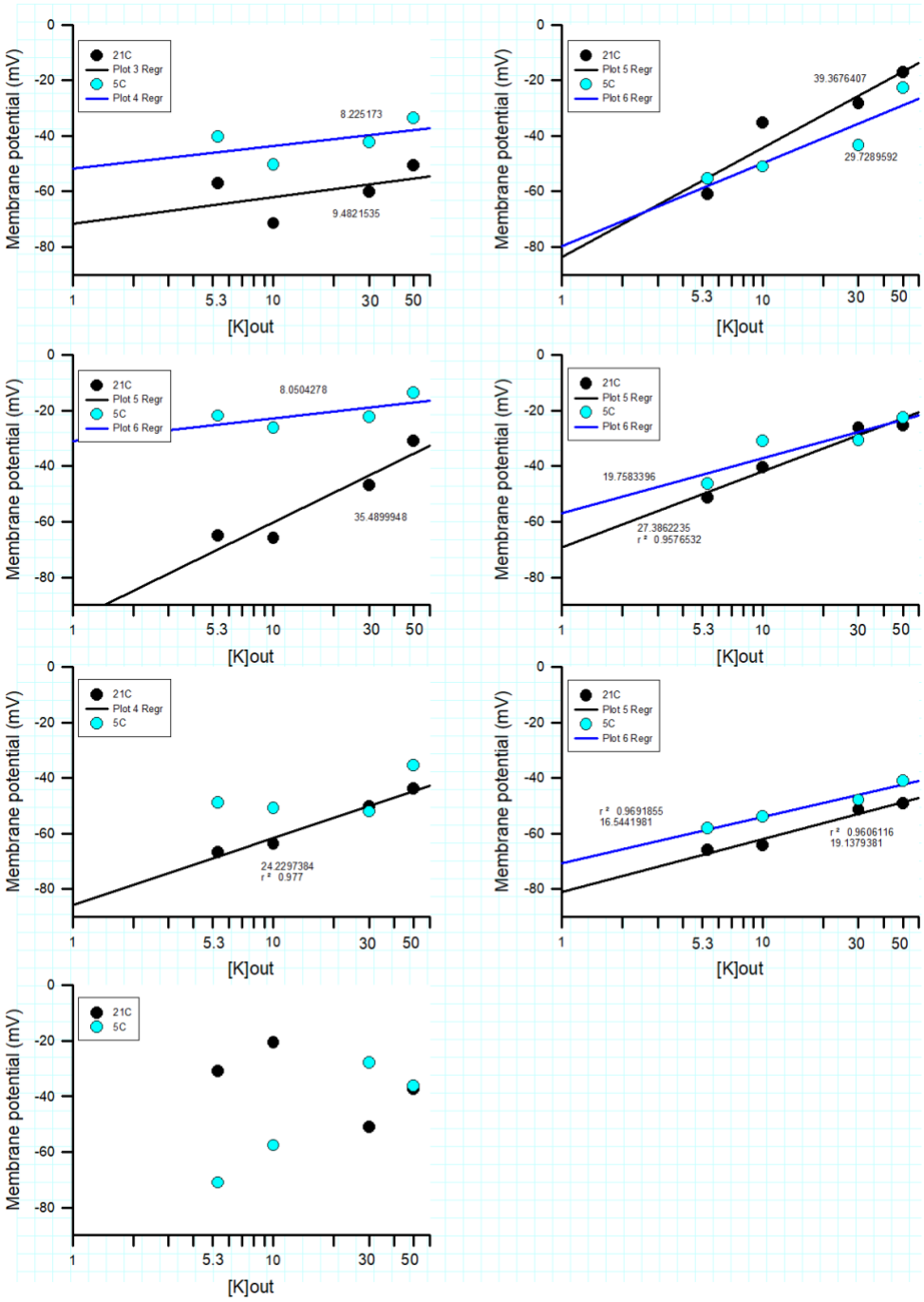
Computational data

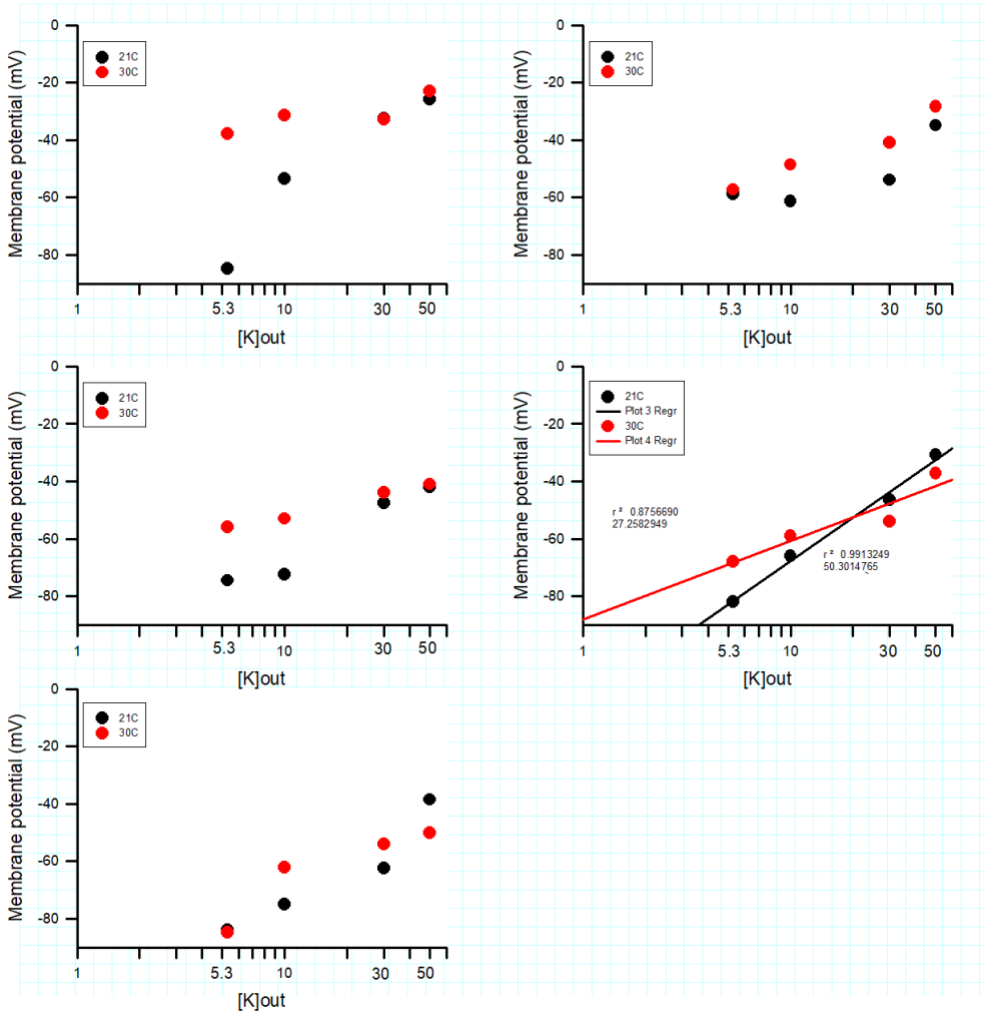
Student obtained graphical analysis of the computational obtained data for four different temperatures and varying $[K^+]_o$. This data is also presented in tabular form in supplemental information for instructors to use as they wish for their classes.



Experimental results

Half the of the class examined the membrane potential at room temperature 21ºC with altering the $[K^+]_{out}$ and again at 5ºC in one lab setting and other half of the class used 21ºC with altering the $[K^+]_{out}$ and again at 30ºC. The individual graphs of each student's results are shown below. Linear regression lines are shown for some graphs of the data which indicated a linear relationship.


21C
Plot 3 Regr
5C
Plot 4 Regr
8.225173
9.4821535
Membrane potential (mV)
[K]out
21C
Plot 5 Regr
5C
Plot 6 Regr
39.3676407
29.7289592
21C
Plot 5 Regr
5C
Plot 6 Regr
8.0504278
35.4899948
21C
Plot 5 Regr
5C
Plot 6 Regr
19.7583396
27.3862235
r² 0.9576532
21C
Plot 4 Regr
5C
24.2297384
r² 0.977
21C
Plot 5 Regr
5C
Plot 6 Regr
r² 0.9691855
16.5441981
r² 0.9606116
19.1379381
21C
5C


Membrane potential (mV)
0
-20
-40
-60
-80
21C
30C
1
5.3
10
30
50
[K]out
Plot 3 Regr
Plot 4 Regr
r² 0.8756690
27.2582949
r² 0.9913249
50.3014765

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit http://www.ableweb.org/.

Advances in Biology Laboratory Education is the peer-reviewed publication of the conference of the Association for Biology Laboratory Education. Published articles and extended abstracts are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board. Published abstracts are evaluated and selected by a committee prior to presentation at the conference.

Citing This Article

Janki Naidugari, Dara Buendia Castillo, Mohammad Abou El-Ezz, Cameron Brown, Tom Calderaro, Cameron Evans, Trey Grant, Rachel Hazelett, Cassity High, Tessa Ilagan, Jack Klier, Nicole Marguerite, Felicia Marino, Shelby McCubbin, Nicholas Meredith, Blair Nethery, William Russell, Noah Sommers, Esther E. Dupont-Versteegden, Rebecca Krall, Katherine Sharp, Jeffrey Chalfant, Mia Brown, and Robin L. Cooper. 2022. Temperature Dependence of the Passive Effects of K^+ on Membrane Potential of Skeletal Muscle as an Educational Module. Article 67 In: Boone E and Thuecks S, eds. *Advances in biology laboratory education*. Volume 42. Publication of the 42nd Conference of the Association for Biology Laboratory Education (ABLE). https://doi.org/10.37590/able.v42.art67