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An Educational Model for Understanding Acute Deep Tissue Injury of Motor Units: Common Lab Exercises with a New Twist

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This laboratory procedure highlights neurophysiology exercises in synaptic transmission at neuromuscular junctions in relation to a practical problem. The exercise is left open-ended in several ways so instructors and students can modify it to tackle new questions. This is an ideal exercise as a course-based undergraduate research experience (CURE) to address authentic research questions. The research hypothesis states that muscle injury would result in a pathological increase in K^+ concentration within muscle tissue, which would affect surrounding healthy cells. This was examined by direct application of saline containing a higher than normal concentration of extracellular K^+ ions. Physiology exercises commonly study the effects of extracelluar K^+ on resting membrane potential. However, this exercise adopts a novel approach by studying extracellular K^+ ions in the context of cellular injury alongside other contributing factors related to injury. Crayfish motor units were used as the experimental model to determine the consequences of damaged muscle on surrounding healthy muscle and neuronal function. The research questions on this topic are based on understanding the physiological problems associated with deep tissue injury of skeletal muscle and/or neurons. Primary skeletal muscle damage can produce secondary effects which can increase the spread of the initial damage zone. This can be caused by the additive effects of intracellular contents, particularly free K^+, released from crushed muscle cells. Sample data is provided for the exercises presented. This educational module can also help establish other animal models which may lead to better treatment and assessment of deep tissue injury in urgent care centers for mammals.

INTRODUCTION

A university-level student laboratory exercise was designed around the theme of deep tissue injuries (DTIs) and related pathological consequences. An invertebrate crayfish model was used which has a long history for college level teaching of neurophysiological principles. To stimulate interest in the growing number of undergraduate allied health focused students, the theme of this laboratory exercise was focused on a medical problem a physician may face when attempting to rehabilitate a patient from a tissue injury to prevent further muscle/cellular damage due to the spread of the initial injury (Brancaccio et al., 2010; Cintra-Francischinelli et al., 2010). The spread of indirect tissue damage from a direct injury is a common occurrence with DTIs.

As an exercise for this student-lead research project, one can assume a relative large mass of tissue is injured (i.e., skeletal muscle) by either blunt force trauma of external object and/or with pressure injury from the internal skeleton. The testable hypothesis is that damaged muscle can cause an altered function of healthy muscle and neurons. In addition, intracellular constituents (i.e., K^+ and amino acids) from injured muscle may also play a role in the spread of tissue dysfunction. Thus, a treatment to obtain a normal extracellular environment can help promote a faster recovery from the initial DTI insult. Students can develop variations to the experimental preparations presented in these laboratory exercises. The preparations presented consist of two types of muscle fibers (slow and fast). These preparations are well-known for student neurophysiology experimentation but novel for the use of investigating an injury topic on muscle and nerve function.

Crayfish preparations are commonly used in undergraduate and graduate classes to teach basic neurophysiological measures (Johnson et al., 2014). The crayfish abdominal extensor muscle preparation is used to demonstrate effects on resting membrane potential with ion substitution in saline and is a good preparation for demonstrating synaptic responses for different types of motor units. Some muscles in crustaceans are selectively innervated by either a phasic or a tonic motor neuron, although some single fibers can be innervated by both phasic and tonic excitatory motor neurons, such as for extensor muscle in the crayfish walking legs (Atwood, 1976; see movie explanation in Wu and Cooper, 2010) and most other limb muscles (Wiersma, 1961a). By selectively

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stimulating phasic and tonic motor neurons, physiological differences in the excitatory junction potentials (EJPs) may be measured. Phasic motor neurons produce rapid twitching of muscle fibers and evoke EJPs on the order of 10–40 mV. The phasic response can depress rapidly with 5–10 Hz trains of stimulation. The tonic motor neurons give rise to smaller EJPs that increase in the presence of a higher stimulation frequency (i.e. 10–50Hz). Structurally, the presynaptic phasic and tonic terminals at the NMJs are fundamentally different (Atwood and Cooper, 1996a; Atwood and Copper, 1996b; Bradacs et al., 1997; Cooper et al., 1998). The topic of muscle phenotype can be presented to the students to investigate if different types of motor units are variably affected by cytoplasm spillage of a DTI.

Using scenarios related to practical issues to help unravel complex biological systems provides students with authentic contexts in which to learn and apply scientific concepts (Coll et al., 2005). Models designed to explore and construct scientific explanations also promote metacognitive thinking, improve communication skills and create opportunities for students to participate in the development of scientific knowledge (Gilbert et al., 2000). These approaches are hallmarks in student retention and understanding of novel concepts. Having exercise modules which can be built upon for novel experimentation is not only important for a course-based undergraduate research experience (CURE) but is also necessary to potentially develop an authentic course-based undergraduate research experience (ACURE; Bakshi et al., 2016; Wycoff et al., 2018). The purpose of this exercise is to learn to answer questions such as: What is DTI? How does excess K^+ affect neuron function? How is this topic going to be looked at in a crayfish model? Why is this a good model to address DTIs in other animals?

The learning objectives which were set out for this exercise are as follows:

1. Students will learn about physiological conditions of membrane potential
2. Students will learn terminology related to membrane potential (depolarization, hyperpolarization, resting membrane potential, voltage gated channels, passive leak channels, permeability)
3. Students will learn the meaning and application of the Nernst Equation and Goldman Hodgkin Katz equation
4. Students will learn the general differences in ionic concentrations across cells and how they are maintained by pumps, exchangers, and other transport mechanisms

A text description of this exercise to distribute to participants enrolled in a laboratory course conducting the experiments is provided in the Appendix. Notes for an instructor teaching this module is also provided in the Appendix. The instructor notes outline potential problem areas students may run into while conducting these exercises.

MATERIALS AND METHODS

Preparation and Dissection

These experiments were all performed using *Procambarus clarkia* (*P. clarkii*) crayfish measuring 6–8 cm in body length (obtained from Atchafalaya Biological Supply Co., Raceland, LA, USA). Each animal was individually stored in an aquatic facility and was fed commercial fish food pellets (Aquadine, Healdsburg, CA)—marketed as "shrimp and plankton sticks: sinking mini sticks"—for at least two weeks prior to experimentation. For invertebrate species, an Institutional Animal Care and Use Committee (IACUC) animal-use protocol approval is not generally required in the USA. However, best practice in reducing any potential stress or discomfort is followed. Crayfish were rapidly placed in an ice slurry for five to ten minutes to quickly reduce neural function. Afterwards, the anterior part of the cephalothorax, which contains the brain, was cut away from the body.

The protocol for exposing the deep abdominal preparation is described in several studies and in protocols for educational purposes (Baierlein et al., 2011; Johnson et al., 2014; Parfitt, 2002; Sohn et al., 2000; Wyttenbach et al., 1999). In brief, the articulating membrane which joins the abdomen and cephalothorax was cut separating the abdomen. The swimmeret appendages were cut away, and a midline cut on the lateral border of each side of the abdomen was made. The caudal telson was cut away from the abdomen and discarded. The intestinal tract along the midline of the deep flexor muscles was removed. The ventral half of the abdomen was removed and the dorsal half was placed in a recording dish filled with standard crayfish saline. Standard crayfish saline is a modified form of a Van Harreveld's solution (1936), which was made with 205 mM NaCl; 5.3 mM KCl; 13.5 mM $CaCl_2 2H_2O$; 2.45 mM $MgCl_2 6H_2O$; 5 mM HEPES and adjusted to pH 7.4. Freshly-dissected preparations were bathed in standard crayfish saline. After the preparation was pinned in the dissecting dish, any lingering damaged muscle fibers from the ventral half of the abdomen were removed. There are two main types of muscles and motor unit groups to examine: the deep extensor lateral (DEL1) or the more lateral deep extensor muscle bundles (DEL2) for phasic muscle and the superficial extensor lateral (SEL) for a tonic muscle phenotype and the associated motor nerve innervation (Figure 1; Sohn et al., 2000; Pilgrim and Wiersma, 1963).

Intracellular recordings of resting membrane potentials

For intracellular recordings, a sharp glass electrode (catalogue # 30-31-0 from FHC, Brunswick, ME, 04011, USA) filled with KCl (3 M) to obtain a 20-40 MΩ resistance was 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 analysed via Scope and LabChart software

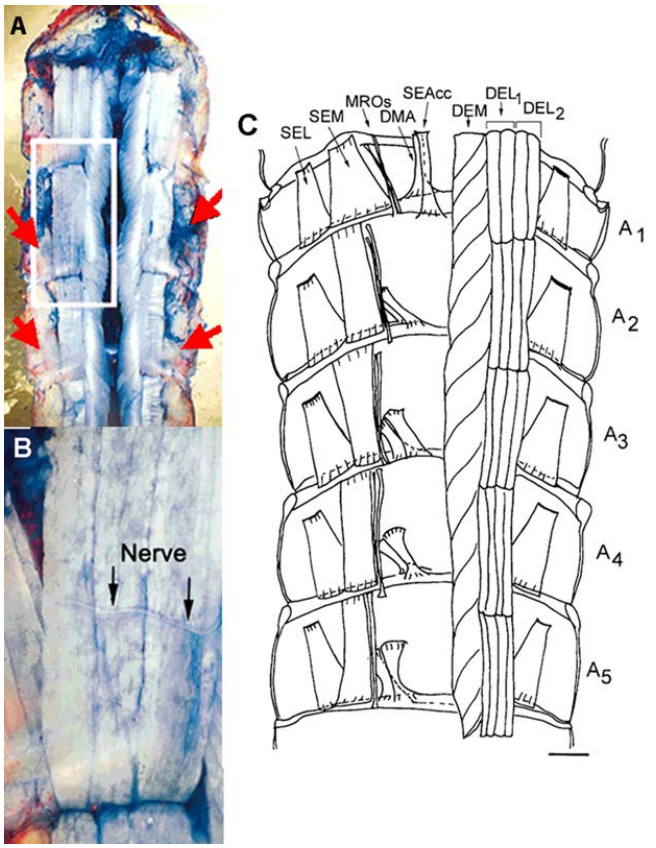

Figure 1. Dorsal part of the crayfish abdomen showing the extensor musculature in the crayfish. (A) The segmental nerve approaches the extensor muscle from the lateral-caudal aspect of each segment. The nerve is close to the SEL muscle. The red arrows depict the approximant locations where the segmental nerve can be located. (B) In this enlarge view the motor nerve can be seen on the surface of the DEL muscle and branching as it heads to the DEM muscle. (C) Schematic drawing from a ventral muscle with labels for the deep extensor medial (DEM), deep extensor lateral group1 (DEL1) and deep extensor lateral group2 (DEL2) used in this study (Scale bar = 2.35 mm; part C modified from Sohn et al., 2000).

(ADInstruments, Colorado Springs, CO). An average value in the resting membrane potential (RP) from three different fibers within the muscle group DEL1 and DEL2 were obtained.

For instructional purposes, prior to exchanging the bathing solutions, participants discussed the following questions to have mindset of the rationale for values in ionic concentrations of the salts used in the experiments and the expected outcomes. The topic was to discuss what a damaged muscle could spill out from the cytoplasm and the potential effects on neighboring muscle and nerve cells. The following questions were discussed: What is the intracellular sodium concentration, $[Na^+]_i$, of cells? How does this alter RP? How would low extracellular sodium, $[Na^+]_o$, and high extracellular potassium, $[K^+]_o$, alter the RP?

The rationale of lowering $[Na^+]_o$ is that with muscle injury the concentration would be diluted if the intracellular fluid of a large muscle is now spilling out into the smaller volume of extracellular space among healthy fibers. In reducing the NaCl in the saline, the

Table 1. Composition of Saline with Reduced $[Na^+]_o$ and Compensated with Choline Chloride.

	100% Na^+ (normal saline)	66.66% Na^+	33.33% Na^+
NaCl	205 mM	136.65 mM	68.32 mM
Choline chloride	N/A	68.3 mM	136.53 mM

Table 2. Composition of Saline with Reduced $[Na^+]_o$ and Compensated with Choline Chloride.

	33.33% Na^+	33.33% Na^+	33.33% Na^+
NaCl	68.32 mM	68.32 mM	68.32 mM
Choline chloride	136.53 mM	136.53 mM	136.53 mM
KCl	20 mM	40 mM	60 mM

osmolarity is maintained by replacing it with the same molarity of choline chloride. However, the osmolarity may change in the extracellular space in an intact animal with an injured muscle. Two different saline solutions, with the various amounts of NaCl and choline chloride, were used for these experiments (Table 1).

After obtaining an average RP in standard saline, the electrode was removed from the muscle fiber and the bathing solution changed to the next saline to be examined. New values of membrane potential were then taken after about a minute for the new saline to mix around the fibers. Upon completing the low $[Na^+]_o$ experiments, the saline was exchange back to the normal crayfish saline in order to use the same preparation for the next series of experiments which used the various $[K^+]_{bath}$. The same process for obtaining the RPs between saline exchanges was used as above. The series of $[K^+]_{bath}$ crayfish saline solutions used were: 5.4 (control), 20, 40, and 60 mM (Table 2).

The above experiments examined changing one ionic concentration at a time, but for the scenario of a DTI for skeletal muscle, or in deep brain region in a mammal, both $[K^+]_o$ and $[Na^+]_o$ would change around the healthy cells because of the damaged cells. In order to examine the effect of both ions changing in the direction which would possibly occur for a DTI, the $[Na^+]_o$ was kept low and the $[K^+]_o$ was gradually raised as shown in Table 3. The preparation was rinsed with normal saline and allowed to regain a normal starting RP.

In the next series of experiments a defined diluted solution of homogenized skeletal muscle was used. In order to make dilutions of homogenized skeletal muscle, a volume of supernatant of the homogenized crayfish muscle was measured and diluted as a 1/4 and a 1/2 dilution with standard saline. For these experiments, a small dish was used so only small volumes of the homogenized crayfish muscle solution was needed for each preparation. The crayfish muscle is homogenized after measuring the volume in a graded Eppendorf tube. The tissue was homogenized with a small mortar and pestle. The homogenized tissue and solution were added back to an Eppendorf tube to be spun slightly (1,000 RPM for 1

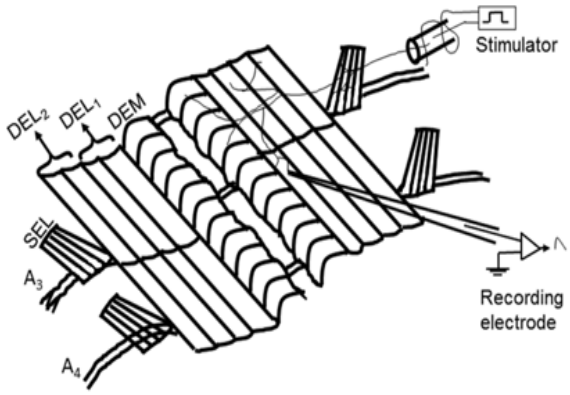

Figure 2. Schematic presentation of crayfish abdomen extensor musculature. Each side of each segment contains deep extensor medial muscle (DEM), deep extensor lateral muscle 1 (DEL1), deep extensor lateral muscle 2 (DEL2), superficial extensor medial muscle (SEM). DEM, DEL1 and DEL2 are fast muscles whereas SEL has a slow muscle phenotype in nature. A3-A4 refers to abdomen segments. (Drawing made by Dr. Yue Chen Zhu).

min). Earlier attempts with needle aspiration back and forth were problematic because the needles would clog, so this method was not preferred.

Measures of synaptic excitatory junction potentials (EJPs)

For ease in implementing the exercises, the resting membrane potentials were first obtained, and then new crayfish preparations were used for examining the effects of the various bathing media on the amplitudes of the EJPs. In the series of measuring the EJPs, another set of RP values were collected which helped to reinforce the results from earlier set of experiments.

To avoid the preparation from large amounts of twitching, the nerve to DEM muscle was transected. EJPs measures are made on DEL1 and DEL2. The DEM muscle may be used if recordings of the RP in various bathing solutions are to be obtained but this muscle was difficult to keep an impaled intracellular electrode while measuring EJPs due to the small fibers and twitching.

The segmental nerve was stimulated with a standard suction electrode set up (ASTRO-MED GRASS stimulator - Model SD9, Pleasanton, CA). See Figure 2 for details and previous reports on methods (Wyttenbach et al., 1999; Sohn et al., 2000; in a video Baierlein et al., 2011; Johnson et al., 2014). Using 0.5 Hz stimulating frequency and turning off the stimulation when not needing to collect data prevented the synaptic responses from fatiguing.

It is interesting to note the phasic motor nerve to the DEL1 will cross a segmental boundary to the more caudal segment, and EJPs can be collected with less muscle movement in this next segment from the stimulating electrode (Figure 2). For observing and measuring the EJPs in the SEL tonic muscle a short train of stimuli maybe needed to facilitate the EJPs. The SEL muscle is innervated by various motor units, so one needs to increase the voltage until the maximum amplitude in the responses are obtained. Started with stimulation frequencies of 2-4 Hz while raising the voltage until EJP responses are observed was a favorable approach.

An average of five EJP amplitudes were obtained for reporting a mean value. For the tonic NMJs the amplitude of the EJPs was taken at the plateau in the amplitudes of the EJPs. The EJPs from tonic NMJs were measured in two fashions: (1) the peak amplitude from the RP and (2) the peak amplitude from the trough between stimuli in case the EJPs are summating on top of each other.

Table 3. RPs in Varying $[Na^+]_o$.

$[Na^+]_o$ (mM)	Membrane potential (mV)
205	-68
136.65	-72
68.32	-75

Table 4. RPs in Varying $[K^+]_o$.

$[K^+]_{out}$ (mM)	Membrane potential (mV)
5.4	-81.9
20	-66.2
60	-31
80	-16

Table 5. RPs with Low $[Na^+]_o$ (68.32 mM) and Raising $[K^+]_o$.

$[K^+]_{out}$ (mM)	Membrane potential (mV)
20	-72
40	-55
60	-40.3

Student assessment

Questions one through five below were given to a freshman class (n = 14) of university level students who have not taken a physiology class in college and have not conducted electrophysiological experiments prior to the self-assessment. All students volunteered for the project as there was no grade or benefit associated with the activity. The questions were given prior to conducting the laboratory exercise and after performing the exercise, with the exception of omitting question number four in the survey after experimentation. The general assessment questions were only provided after completing the exercise. The results were not recorded with participant names or coding and the results were not matched for prior and after conducting experiments. Only an aggregate of the data was recorded before and after conducting the experimental exercise.

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. Ca^{2+}
   B. Na^+
   C. K^+

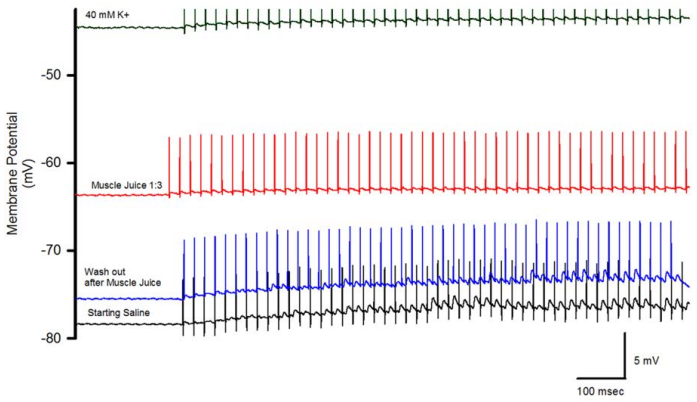

Figure 3. Relationship in size of the EJPs and membrane potential for muscle homogenate (one part muscle homogenate and three parts saline) and 40 mM $[K^+]_o$ from SEL muscle. Note only stimulus artifacts are present with the muscle homogenate exposure but with 40 mM K^+ EJPs are still observed even with a more depolarized resting membrane potential.

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 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 experiment?
   A. Yes
   B. No

5. If one damages a small mass of tissue in the central nervous system inside the brain (ie. maybe a blood clot cuts off some oxygen supply for a while and the cells die) what might be the consequences to neighboring healthy cells that did not die from the low oxygen (short answers)?

General assessment questions

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

Table 6. RPs with 1/4 dilution, 1/2 dilution, and 100% Muscle Homogenate.

Muscle homogenate	Membrane potential (mV)
1/4 dilution with normal saline	-77 - (-67)
1/2 dilution with normal saline	-55
No dilution only supernatant	-31

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 the definition of deep tissue injury
4. The videos that accompanied this exercise helped my understanding of topics discussed
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

RESULTS

Resting membrane potential changes with lowered Na^+ and/or raised K+ in the bath

To determine the potential changes in the RP from a DTI injury of muscle tissue where the $[Na^+]_o$ would be diluted, the RPs hyperpolarized (Table 3). However, in keeping $[Na^+]_o$ at 205 mM and increasing the $[K^+]_o$, the RPs depolarized (Table 4). To examine the effect of both ions changing in the direction which would possibly occur for a DTI, $[Na^+]_o$ was kept low and the $[K^+]_o$ was increased, resulted in a reduced depolarization as compared to only raising $[K^+]_o$ (Table 5).

In the next series of experiments the diluted solution of homogenized skeletal muscle was used for comparing the RP values obtained with altering the specific ions (Table 6). As the amount of homogenate was increased the membrane became more depolarized.

Synaptic excitatory junction potentials (EJPs)

The EJPs of the SEL tonic muscle, during a short train of stimuli at 50 Hz, facilitate in amplitude and quickly reach a plateau level (Figure 3). During the various trials of switching out bathing solutions it was determined the RP would recover closer to the ini-

tial values if the experiments with the muscle homogenate were completed first and then rinse the preparation with standard saline before starting the next series of experiments with combined low $[Na^+]_o$ and varied $[K^+]_o$ trials. Representative responses are shown in Figure 3 from the SEL with normal saline, muscle homogenate at 1:3 (one part muscle supernatant to three parts normal crayfish saline) followed by a normal saline wash and then a 40 mM $[K^+]_o$.

Quantitative and qualitative responses from students

These sets of experiments were conducted with participants in a freshman class and group discussion took place after the experiments were complete. Prior to conducting the exercises, a survey was given to them and after conducting the exercises. With the knowledge gained on the electrochemical gradients of ions in this exercise, the students were able to discuss the actions of injured cells on the neighboring healthy cells. As potential to human health, the students engaged in some of the following questions: What might be an approach to reduce the damaging effects with a DTI? What other factors in relation to the muscle contents might need to be considered, which have not been addressed in isolation in these sets of experiments mimicking a DTI? Some students asked family physicians and sought out answers to these questions on their own. A practicing vascular surgeon as well as a certified practicing wound care specialist (certified nurse) were also asked such questions in context of knowing it was for a class project. The standard treatments discussed were to insure IV saline flush in considered to rid the body of the excess K^+ and protein. The potential for acute renal failure is also monitored due to proteinuria. The main issue to be clinically concerned with is the rising $[K^+]_o$, and thus it would be monitored often for a patient. Students posed what-if questions and one of the instructors repeated the questions to practicing clinicians: "What would be the treatment if a large DTI of skeletal muscle occurred for example in the thigh or calf without the skin being compromised?" The answer was the same as the standard clinical protocol mentioned above. "What if the skeletal muscle of injured area could be flushed with an IV saline type of flush but directly inserted around the damaged muscle and a drain catheter could be inserted to remove the excess saline being flushed in along with the cytoplasmic spillage around the healthy cells?" The clinician's response was, "This sounds like a practical and convincing idea." The students became ecstatic when the responses were reported back to them. The students conveyed they felt these particular laboratory exercises, which are relatively inexpensive to implement, highlighted the usefulness of model invertebrate preparations to address fundamental questions.

Student assessment reports and analysis

The results of the student self-assessment reveal that there was little understanding of the main concepts prior to conducting the experimental procedure. However, the concepts were well understood after performing the exercise. The answers to the selections before and after the exercise for one through five (omitting question four in the post survey) are shown in Figure 4. The responses to the rubric for question five are also included. It is important to focus initially on the responses for question four which asked if

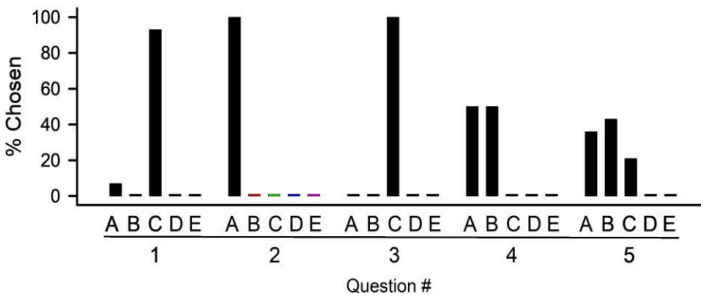

Figure 4: The results of the participant survey on content questions taken before and after conducting the exercise. The numerical responses are shown for each of the answers. The coded rubric A through E for question five is included (see rubric).The small bars at the 0% are drawn in at the 1% level only for reference to illustrate the pre- and post-responses, but the values are 0%.

the student had any prior background with the Nernst equation or the Goldman-Hodgkin-Katz equation. The majority (86%) had no prior exposure to these concepts of resting membrane potential. This helps to illustrate the lack of knowledge in the responses for the pre-test question one in knowing if K^+ is of a higher concentration inside cells as compared to the extracellular fluid. However, note the response for question one after the exercise in which there was a large improvement in understanding of this content. The responses for question two is not surprising as this particular cohort of students have been working with Drosophila melanogaster (fruit flies) in monitoring heart rate and behaviors related to physiological stressors for four weeks prior to this exercise. The responses on the post-test for question three showed a large improvement as well after conducting the exercise. What was impressive was the significant improvement in details for the open responses related to question five. Students had a much more thorough understanding of the concept that when cells are damaged not only is K^+ spilling out, but also proteins and amino acids, which can alter synaptic transmission.

The rubric used for assessing open responses for question five was developed prior to reading the responses but was constructed broad enough to capture exemplary responses in the top ranking and a range of response throughout. The rubric used is shown below.

Rubric used for question five is as follows:
Question 5: If one damages a small mass of tissue in the central nervous system inside the brain (i.e. maybe a blood clot cuts off some oxygen supply for a while and the cells die), what might be the consequences to neighboring healthy cells that did not die from the low oxygen (short answers)?

A Scientific & in-depth response:

- When a nerve cell is damaged and higher than normal K^+ level and amino acids as well as free proteins. These compounds are not normally high around the outside of the neurons. This can depolarize the neighboring healthy cells and may lead to

death of the neighboring healthy cells. The high amino acids and proteins may also interfere with receptors for the neurotransmitters and enzymatic processes
- Includes scientific justification for selecting correct response and no other extraneous information

B Scientific, but not in-depth:
- The cell debris may harm the neighboring healthy cells. The neighboring healthy cells may include neurons and muscle cells and supportive cells. Maybe the neighboring healthy cells will use the cellular debris as energy sources

C Partially scientific (scientific fragments) with no non-scientific conceptions
- It is possible the capillaries of circulatory system will take up the dead cell parts. Maybe the dead cell parts will harm the neighboring healthy cells but I am not sure. Tangential response containing no non-scientific conceptions with accompanying correct information

D Partially scientific (non-scientific fragments) with non-scientific conceptions
- Some portion of the answer (either forced-choice or part of written response) is incorrect
- Includes some inaccuracies or conceptual misunderstandings in rationale (e.g., incorrectly states mitochondria will still produce energy while outside the cell in the surrounding fluid to help neighboring healthy cells)
- Rationale may include correct scientific reasoning for some aspects but not directly related to the question
- May mention, but does not elaborate on the consequences of the intracellular constituents neighboring healthy cells

E Non-scientific rationale
- Inaccurate or non-scientific justification or use of graphic rather than scientific reasoning to justify response
- Incorrect explanation
- Illegible/Non-codable/no response

Table 7. Results in participant feedback after conducting the exercise. The data represents the questions one through ten of the general assessment questions. The scores are listed as % of total, (N=14) for (A) Strongly agree, (B) Agree, (C) Neutral, (D) Disagree, (E) Strongly disagree.

	1	2	3	4	5	6	7	8	9	10
A	50	21	29	50	14	7	50	79	36	50
B	50	29	57	36	43	43	29	7	43	29
C	0	36	14	7	29	50	14	14	7	21
D	0	14	0	7	14	0	7	0	14	0
E	0	0	0	0	0	0	0	0	0	0

- Response does not make sense

The responses to the general assessment questions (listed in Methods) are highlighted in Table 7. The response from the participants are very favorable for learning the concepts and the 3-diminsonal learning of practices, crosscutting concepts and disciplinary core ideas (Krajcik, 2015; Achieve Inc., 2013).

Feedback from past students in a neurophysiology upper level course

The voluntary feedback by university students who have conducted these laboratory exercise after a school year was completed have been positive and demonstrate they liked the practical nature of the exercises. These students had an entire semester of a neurophysiology laboratory experience. Some of the comments are as follows:

"This lab provided students the opportunity to utilize their understanding of potassium's effect on nerve cell conduction in the crayfish and transmit this to predict the outcome of muscle juice on nerve cell conduction. We were able to apply our knowledge of cells high intracellular potassium to predict what would occur when muscle juice was applied based on our initial findings with saline potassium. The practicality of this lab made it extremely effective in enhancing my learning through applied knowledge and skills of neurophysiology."

"The experiment is very helpful for a student to transfer knowledge from text books to real life experience and skills. It allows us to understand the importance of potassium in the resting membrane potential. It deepens our understanding for the Nernst equation, since we can predict different resting membrane potentials by using different potassium concentrations in the Nernst equation. More than that, the experiment also provides a unique perspective on possible clinical scenario on muscle injury as we were testing muscle juice impact on resting potential. Personally, I found this experiment enlightening and inspiring. The math model for resting membrane potential is abstract but it comes to make sense when we practiced them in this animal model and it really encouraged us to think more about its other applications on clinical scenario and basic physiology foundations for organism to be functional."

"This unique procedure allowed the students to examine the general physiological properties of excitable membranes and the role of a particular ion, potassium, in altering resting membrane potential. From a physiological standpoint, the study allows one to address the consequences of increasing extracellular cation concentration on neighboring healthy cells. This holds clinical relevance in the case of deep tissue injury, which can progress as intracellular stores from damaged cells are released. Furthermore, the role of increasing extracellular K^+ concentration on synaptic transmission in surrounding cells, including the potential Na^+ or Ca^{2+} channel inactivation as a result of altering RP can be assessed. I found this experiment to be particularly useful in enhancing skills that pertain

to basic neurophysiological studies. It promotes the importance of animal models in understanding basic principles that may be clinically significant and its practicality allows one to enhance knowledge of the subject matter that corresponds with teachings from the textbook."

"The lab was helpful in demonstrating how varying extracellular ions affect membrane potentials and synaptic responses. Using damaged muscle as the source of these extracellular ions also helped to exemplify a few of the many abstract concepts of neurophysiology."

DISCUSSION

This report highlights a significant modification to a standard neurophysiology laboratory exercise using an approach of a practical thematic topic. Included in the teaching exercise are the basic concepts in the driving factors which produces membrane potentials and alterations in synaptic transmission. The novelty of using DTI as a theme opened up some new approaches of implementing this laboratory exercise of only altering the $[K^+]$ in the bathing media. Lowering the $[Na^+]$ and raising the $[K^+]$ mimics closely to the environment induced by a DTI. The additional experiment of using a muscle homogenate directly addressed the type of responses induced by acute cytoplasmic spillage on healthy cells.

In one class, the analysis of the data was comprised of having the participants plot the measures obtained for the resting membrane potentials at each of the various ionic concentrations they tested. They also plotted the hypothetical values for only altering the $[K^+]_o$ to determine if the alteration in $[K^+]_o$ alone could explain the observed results. The graphs for varying $[K^+]_o$ are shown in several publications (Atwood and Parnas, 1968; Baierlein et al., 2011; Wyttenbach et al., 1999) so students will have access to those publications. However, as far as we are aware the novelty for the participants in these experiments is the effects on RP from low $[Na^+]_o$ or the combined alterations in $[Na^+]_o$ and $[K^+]_o$. Thus, we have not presented a graph in this report as to provide a unique learning experience for web savvy college students of today who would find the published graphs.

Future participants conducting these experiments can download free graph paper online or even plot on Excel or a graphing program quickly. The tables have limited sample data entrees from participants conducting these exercises for comparisons. After the participants in this exercise completed the graphing analysis various types of questions were poised to them as follows:

In altering the external level of altered ions, did you expect the same type of alterations in the RP and EJPs as observed for changing the external level K^+ concentration alone? How well did the raised $[K^+]o$ match the muscle homogenate dilutions for the RPs and EJPs? Were the changes as expected knowing the concentration of $[K^+]_i$ inside of cells (~120 mM)?

In the open response, the second survey question (see Methods) about improving one's understanding the scientific method by introducing the experimental project as an investigative approach instead of informing the students of the experiment was viewed as a positive experience. Also, letting the participants contemplate various way to go about determining the effects of cell damage on healthy neighboring cells and then guiding the students to an approach using the crayfish or a frog skeletal muscle experimental design to address the questions kept students engaged. The responses for the fifth question (see Methods) would likely have improved by introducing the experiments as hypothesis testing instead of the providing the key elements of raising $[K^+]_o$ and lowering $[Na^+]_o$ for the experiments. The participants who selected that they disagreed in feeling more comfortable in developing a muscle fiber electrophysiology experiment were likely individuals who were new to the instrumentation and software for the analysis of the recordings. This cohort was introduced to measuring membrane potential with intracellular recording, new software and the experimental design of altering $[K^+]$ as well as muscle injury all in one laboratory setting. If this experiment was accompanied with a lab in a prior week in which students only recorded resting membrane potentials and became familiar with the software, then adding the muscle homogenate and measuring synaptic potentials in later laboratory meetings, the students would have been less intimated by instrumentation and the complexity the second time around.

In addition, by reading the literature provided with the exercises, students learned that as early as 1902 Bernstein was dealing with the issues of a RP in the axon of a squid. The students were able to see how the early ideas and observations of Berstein (1902) and Nernst (1888) later influenced research in membrane physiology from reading material (Malmivuo and Plonsey, 1995). As instructors for such an exercise, one can emphasize that there are still breakthroughs being made about ion channel function and properties of biological membranes that are very significant in understanding the cellular physiology, which relate to the function of tissues, organs and systems (Nicholson and Hrabětová, 2017). It is common in allied health articles to discuss a topic, such as hyperkalemia, without stating the mechanism of the physiological consequences. The way the experiments are presented in these exercises will help with this issue. Students will be exposed to how hyperkalemia effects muscle and synaptic transmission in a similar manner from crustaceans to humans.

Future experiments

Extensions are readily able to be made in the general experimental paradigm to include the influence of temperature and simulated muscle injury to determine if cooling or heating the muscle shows different effects on the exposure to muscle homogenate which relates to the types of treatments humans use for pressure injuries of skeletal muscle (i.e., DTIs). Additionally, pharmacological agents can be applied to see if they would help or worsen the effects of the spilled intracellular constituents on healthy neighboring cells. Depending on the severity of a DTI, the blood (hemolymph in the case of an invertebrate) will be tainted with the intracellular constituents of the damaged tissue and thus expose neural tissue, which can influence its function. In order to examine such pathological conditions, an additional preparation using a crayfish could be presented to the students with the same theme such as with a

sensory-CNS-motor nerve circuit (Strawn et al., 2000; Inam et al., 2014; Johnson et al., 2014; Weller et al., 2015).

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