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# Exploring mechanisms in a medical treatment for a disease: A teaching/learning module

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Many undergraduate students majoring in biological sciences are interested in professions within health care. To spark their interests in learning physiological concepts, this exercise focuses on an active form of health care and a treatment which is not fully understood. Students will learn that medical care is continuously evolving from evidence-based practice and scientific understanding. Studying the potential mechanisms of pharmacological actions on physiological function may lead to developing a more precise mechanistic understanding that can lead to more precise treatments. Utilizing the primary research literature, fundamental physiological concepts, and client outcomes from case studies, instructors may construct a teaching and learning module based on the content provided. In this regard, the actions of how 4-aminopyridine (4-AP) helps to alleviate some of the rigidity and movement of limbs in people with multiple sclerosis (MS) is explored. It is well established that 4-AP blocks a subset of voltage gated potassium ($K^+$) channels, but it is somewhat counterintuitive how this promotes better locomotive movements. The mechanisms of action from clinical doses may be more related to the physiological changes that occur due to the progression of MS or even by actions on other cells besides neurons, leading to secondary actions on neurons. With the use of inexpensive electrophysiological instruments (i.e., Backyard Brains), invertebrate nerves will be recorded while exposed to 4-AP to directly observe the effects on electrical activity. In this module, multiple physiological concepts are used to construct mechanistic explanations of the phenomenon. The learning objectives are to: (1) become familiar with the basic neurophysiological principles of electrical signals, synaptic transmission, and pharmacological actions of ion channels; (2) demonstrate the disease process of MS, and (3) develop scientific literacy from a review of research studies on the physiological phenomenon.

**Keywords**: inquiry-based learning, medical, neurobiology, neurophysiology, invertebrate

## Introduction

Treatments for diseases usually involve pharmacological interventions. If the health outcomes are beneficial, the interventions are maintained for treatment and sometimes are examined for other disease states which may not have been originally targeted. In some cases, the medication may be used even without fully knowing the mechanism of action which improves physiological function. To design more effective medications and potentially reduce side effects, understanding the mechanism of action is important. Still, knowing the action of a compound on function in a particular tissue or how the compound may affect cellular processes may not fully explain how the compound is causing the improvement in a complex body involving many systems interacting together. In a disease state, the conditions known or expected for the action can be varied as the compounds may not have been experimentally addressed within a pathological state.

In this report, we highlight an educational module addressing the potential mechanism of how 4-Aminopyridine (4-AP) can be beneficial for some patients with multiple sclerosis (MS) (Baker 2013; Correale et al. 2017). This is an educational module which builds on the basic knowledge known about how the compound works on cells. Participants are to understand the basics of how neurons are electrically excitable and conduct electrical signals as well as communicate with other cells. The module addresses the various ion channels and ionic regulation of electrical events as well as the process of chemical synaptic transmission before diving deeper to examine the different types of voltage gated potassium ($K^+$) channels and how they can modulate the shape of an action potential, influence membrane excitability, and alter synaptic transmission (Clay 1985). It is well established that 4-AP blocks a subset of voltage gated $K^+$ channels.

In addition, the module highlights the different progressions of MS and the various effects on the nervous system. Identifying the complex pathology of the disease and introducing how neurons are altered by the disease will highlight how 4-AP may impact physiological function in various cell types (Krishnan et al. 2013; Jensen et al. 2014; Filippi et al. 2020). This content allows the participants to then postulate how 4-AP can enhance locomotion in some people with MS.

A review of the primary literature will be used to postulate the mechanism of action of 4-AP on physiological function. The controversies in the primary literature are introduced using clinical data and reports from experimental research on animal models (see Bagchi et al. 2014; Jensen et al. 2014). The latest understanding with sound scientific arguments will be presented to explain how 4-AP is beneficial in this disease state as well as what is still in question in the understanding.

This exercise teaches the fundamental principles of the scientific process. A review of various resources highlights the historical views and how they change over time as the scientific knowledge base develops. Further, the investigation teaches how the scientific process proceeds and how ideas are refuted with evidence. Lastly, one can conclude that it is hard to obtain a complete understanding as still more questions arise, requiring continued research (see Anderberg et al. 2007).

# Student Outline

## Objectives

Describe the disease state of multiple sclerosis.
Describe current treatments to alleviate the symptoms of multiple sclerosis.
Learn how to dissect a preparation to be able to make physiological recordings.
Learn how to record extracellular electrical activity from a nerve.

## Introduction

*Part A: Literature-based content*
This is explained in a Youtube link: https://youtu.be/6s4NK39fEBo

Case Example:

You and your classmate, Jane, have been studying for your spring semester final exams. Jane tells you that she has been experiencing symptoms such as tripping over her feet, weakness in her legs, double vision at times, and feeling overall fatigued. She contributes this to staying up late studying and completing projects in school as well as working longer hours at her off-campus job. She feels confident that before the fall semester, she will be able to rest and then go back to feeling normal. On the first day of the fall semester in physiology class, you notice classmate Jane walking slower and having trouble going up the steps in the classroom. She appears exhausted upon sitting down next to you and reports she is experiencing more "pins and needles" in her legs but that she is working with her primary care provider to determine the cause of these symptoms. The following week, she comes to you and tells you that she has been diagnosed with Multiple Sclerosis (MS).

She states she felt overwhelmed during the doctor's appointment and was hoping that you would be able to help her better understand the pathology involved with MS. Due to her difficulty with walking, the provider prescribed her a medication called Ampyra (dalfampridine) extended-release tablets (4-AP). She is wondering if you can also help her understand how this drug is supposed to help with her walking. Since the diagnosis, she has joined a support group that includes members of all ages and different types of MS and has talked to others that have taken Ampyra. She states some of the other members did not report any improvements in their walking when taking this drug.

To watch an example of how the medication can influence walking, go to https://ampyra.com/real-patient-videos.

To answer the following questions, use any reference material provided (textbook, PowerPoint slides, and/or the YouTube video linked above).

1. Explain basic neuron function including excitability and synaptic transmission
    a. Explain resting membrane potential and how different ion channels and ion permeability alters membrane potential (Nernst and Goldman-Hodgkin-Katz equation).
    b. Draw out an axon to explain electrical excitability and electrical conduction. Two types of neurons: a myelinated one and an unmyelinated one.
    c. Draw out a nerve terminal to address chemical synaptic transmission and the process involved related to ionic channels.

2. Explain how various types of K channels are responsible for the shape of the action potential.
    a. Draw out the action potential after Ampra (4-AP) is used and how K channels contribute to the different parts of the action potential.

3. Address the pharmacology related to the various channel subtypes and how different neurons respond differently to the pharmacological compounds.
    a. Draw out the shapes of the action potentials and label how they are altered by the pharmacological compounds (including how the altered shape could influence the voltage-gated calcium ($Ca^{2+}$) channel in the presynaptic nerve terminal).
    b. Draw how the difference in $Ca^{2+}$ loading alters chemical synaptic transmission.

4. Detail the clinical appearance of the disease and epidemiology (how many patients affected, age, male/female, Caucasian, etc.).
    a. Explain the mechanism behind MS (destruction of myelin, disruption of the blood brain barrier).
    b. Explain how histology helps in identifying the inflammation of MS.
    c. Explain how a diagnosis of MS is made via MRI using McDonald Criteria from 2017.

5. Postulate how 4-AP may benefit patients with MS from the basic knowledge addressed above.

6. Address how the pathology of MS may complicate the concepts of how 4-AP may work in the diseased state.

7. Address the concepts presented in the literature and views of the potential actions of 4-AP in MS as well as the controversies presented in the literature.

8. Based on the latest literature, address how 4-AP is mechanistically working to improve the symptoms of MS and why only a subset of people with MS benefit from treatment. Relate your answer back to the initial case/patient description above.

9. List what might be the next research steps required to address how 4-AP might be working to improve MS.

*Part B: Nerve recording with application of 4-AP*

The electrophysiological recordings from a model nerve preparation can be accomplished with various animal models. The instructor for the course will determine which preparation model to use. Below is the procedure for using the crab leg proprioceptive nerve. You can also follow the directions with the video link here: https://youtu.be/dnOfB-K3gyo

1. Cut along the cuticle of the crab leg in each segment to make windows to visualize the muscles and to expose the nerve. See Figure 1. Then place this prep in the Sylgard coated dish (1/2 to ¾ of an inch thick of sylgard) containing saline.

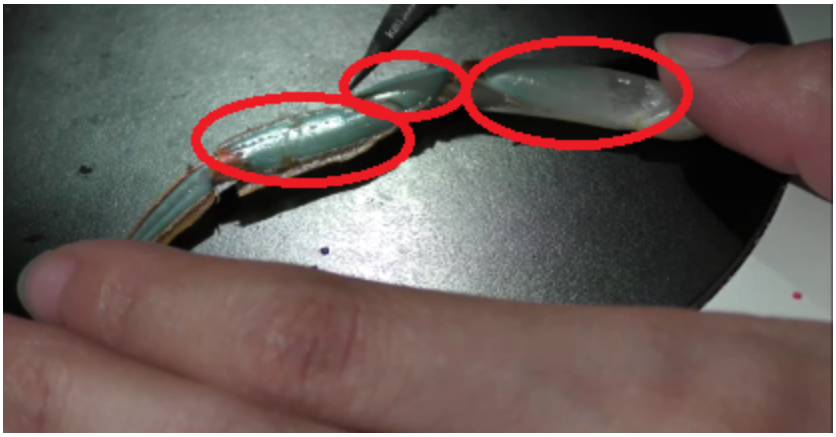

**Figure 1**. The three segments in which the cuticle is cut highlighted by the red circles.

2. Place pins on the dorsal edge of the cut windows to hold the preparation down. Remove the opener muscle (and the muscles on the side of the windows that were made) by cutting the connective tissue holding the tendons (apodemes in arthropods). See Figure 2.

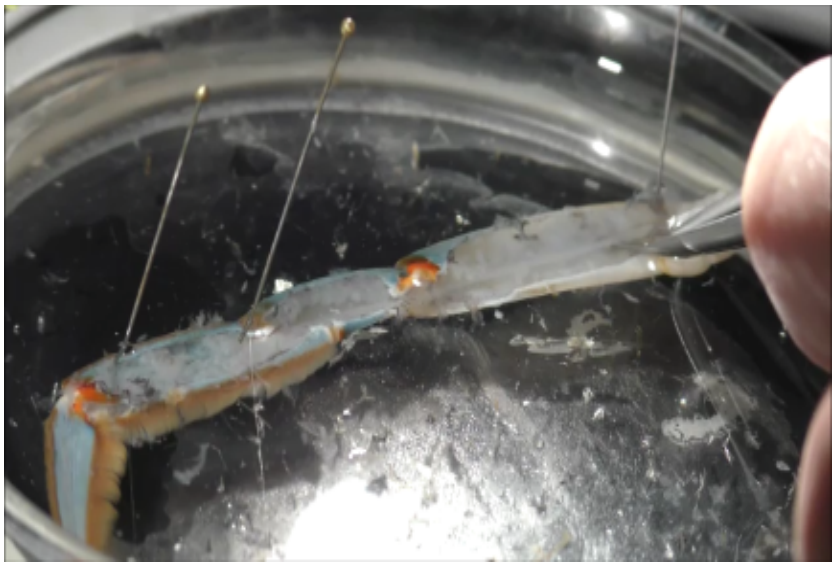

**Figure 2**. Pins are in place to hold the preparation down. Muscle has been cut and now exposing the nerve. The tweezers in the figure highlight the location of the nerve.

3. With the nerve now exposed, cut close to the proximal end of the leg. This cut end will be put in the suction electrode. See Figure 3. Once the nerve is cut, you can cut away and remove the proximal end of the leg segment to make more room in the preparation dish. See Figure 4.

**Figure 3**. Cut the nerve where the tweezers are shown.

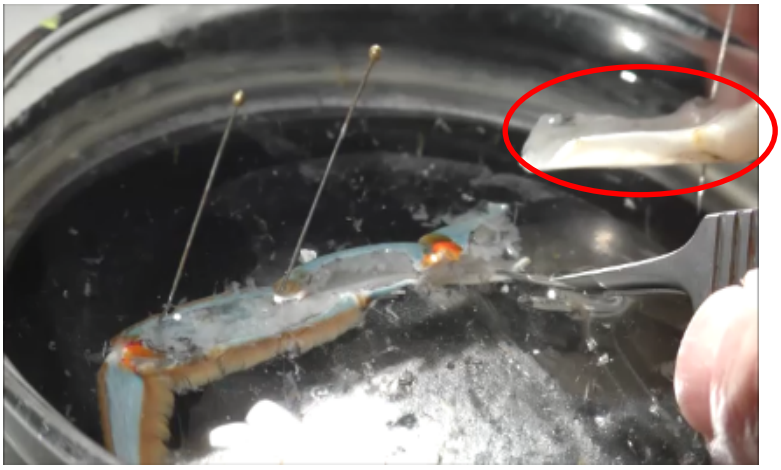

**Figure 4**. The proximal end of the leg is removed (red circle) with the nerve still intact to the remaining leg.

4. Now that you have more room in the preparation dish, you may need to move and re-pin the leg in order to be able to move the distal segment. If you have room to move the distal segment, then there is no need to reposition the leg. Place wax on the outside of the preparation dish to hold it in place. Before pulling the nerve into the suction electrode, saline is drawn into the suction electrode above the wire inside the electrode. The nerve is then gently pushed over to the suction electrode and pulled into the suction electrode. Make sure the saline is covering the wire on the inside of the suction electrode and the outside wires of the suction electrode. See Figures 5 and 6.

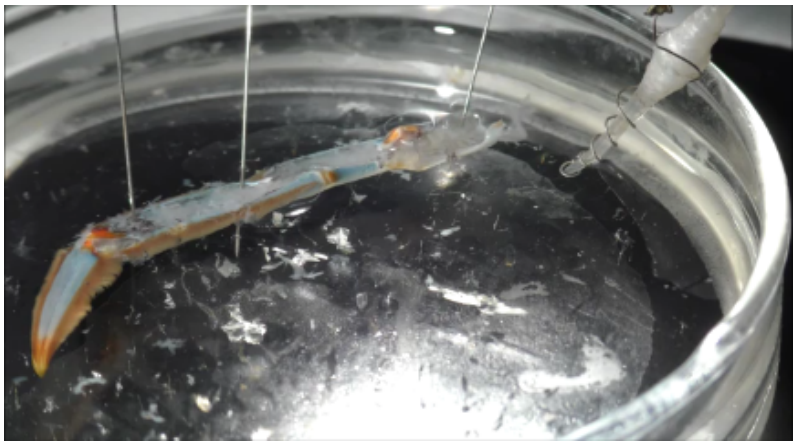

**Figure 5**. Saline is drawn into the suction electrode and positioned near the nerve.

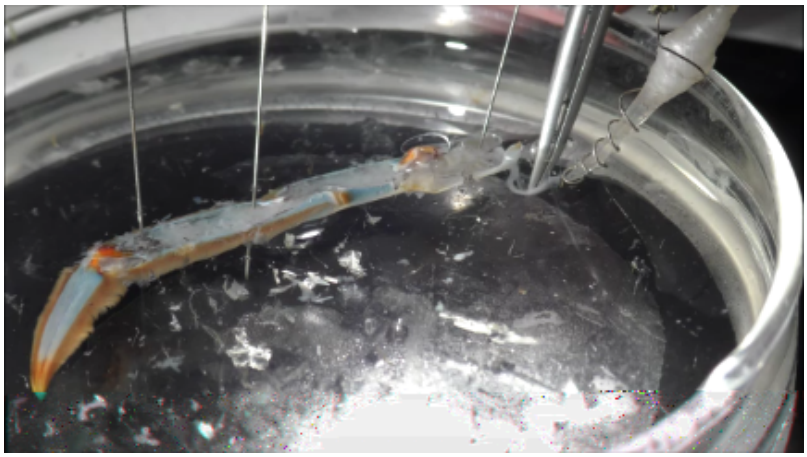

Figure 6: The nerve is gently pushed over to the suction electrode and pulled into the suction electrode.

5. Now that your preparation is ready, move the distal segment of the leg to obtain electrical responses. Repeat several times and save the file for baseline data.
6. NOTE: 4-AP is TOXIC TO HUMANS. Use nitrile gloves. Now switch the saline with saline containing 4-AP and swish around the preparation dish.
7. Repeat moving the distal segment of the leg to see if frequency and amplitude are altered. Save file.
8. Rince the preparation with fresh saline without 4-AP a few times while swishing the saline over the nerve. Repeat the joint movements to see if the nerve activity is recovered.

## Materials

A computer with Internet access is required for each student.
Materials for 1 set up:
Sylgard Dow Corning 182 silicone kit
Dissection dish with ½ inch (2 cm) thick coating on bottom (1)
Dissecting tools (Scissors and a pair of forceps) World Precision Instruments
Dissecting microscope (50X) or self-construct as shown in movie
Fiber optic lamp or self-construct as shown in movie
Micromanipulator World Precision Instruments MD4-M3-R
Raised preparation stand
Silver wire (10/1,000 inch) A-M Systems 782500 if one wants to construct their own suction electrodes.
Amplifier/Acquisition System or can use BackYard Brains set up Spiker Box.
Dissection pins (4)
Crab leg (1)

Saline solution for Blue Crabs :
(in mM: 470 NaCl, 7.9 KCl, 15.0, $CaCl_2 \cdot 2H_2O$, 6.98 $MgCl_2 \cdot 6H_2O$, 11.0 dextrose, 5 HEPES acid and 5 HEPES base adjusted to pH 7.4).

NaCl Sigma-Aldrich S7653
KCl Sigma-Aldrich P9333
$CaCl_2$ Sigma-Aldrich C5670
HEPES acid Sigma 3375 Free acid, crystalline
HEPES base Sigma
D-Glucose Sigma G7021
$MgCl_2 \cdot 6H_2O$ Sigma 246980
NaOH Sigma-Aldrich 221465 To adjust pH
HCl Sigma-Aldrich H1758 To adjust pH

## Notes for the Instructor

This module used the crab leg proprioceptive nerve to obtain electrophysiological data. This model preparation can also be recorded for a teaching lab with high-end equipment and a computer to acquire the electrical signals (see Majeed et al., 2013), or with the use of Backyard Brains equipment for a lower cost version to record electrical activity. However, the lower cost version will require one to make a suction electrode, design a dissecting microscope, and a simple manipulator to move the suction electrode. When using the Backyard Brains devices, be careful as the audio device will pick up sounds, such as talking, which can contaminate the electrical recording of the nerve. The Backyard Brains is a cheaper approach if access to extracellular amplifiers is not available. However, commercially bought extracellular amplifiers for physiology labs generally have filters which can be helpful to clean up the signals and to amplify the signals.

The YouTube link provided to the students (https://youtu.be/dnOfB-K3gyo ) presents the above procedures on how to expose a nerve of the crab leg and record from the whole nerve using the suction electrode and the Spiker box from Backyard Brains. The following are YouTube video links for the set-up prior to experimentation: Sensory nerves in a leg of a crab/lobster/crayfish: https://www.youtube.com/watch?v=yIT2rCvIUoo; Recording nerve activity with a suction electrode & Backyard Brains Part 1of 2: https://youtu.be/LrHXLF96d8Q; Use of suction electrode to record from nerves in saline with Backyard Brains devices: https://youtu.be/kGMBelEapGk.

Generally other crustaceans can be used for this experiment. However, the Blue Crab is widely accessible in many areas along the East Coast of the North America and the Gulf of Mexico. The Dungeness crab (*Cancer magister*) can also be used but the cuticle is thicker and harder to cut the windows with a scalpel. One might have to use a dremel drill with a saw blade. Most major hardware stores supply this equipment. However, care is needed to prevent the saw blade from penetrating too deep as it is easy to damage the nerve. If you prefer to use another animal preparation, the following could be used instead of the crab leg:

1. The frog sciatic nerve preparation is well documented and detailed laboratory procedures can be found online and in educational protocols from companies that sell the associated equipment. Here are some listings:
   (A) The general learning objectives and content are presented in protocols already freely available and readily accessible in multiple links. https://www.adinstruments.com/lt/neuroscience . Then click on "Frog Nerve "
   (B). Iworks even presents that lab in video format https://www.youtube.com/watch?v=YUzne-KZ4_4
   (C) Biopac manual. https://www.biopac.com/curriculum/a03-frog-sciatic-nerve/
   (D) Teaching labs. https://www.medicine.mcgill.ca/physio/vlab/Other_exps/CAP/prep.htm
2. The crayfish muscle receptor organ can be accomplished with high-end equipment as well as inexpensive equipment, but the latter is not as sophisticated for data analysis. See the following for a teaching lab with research-grade equipment and a computer to acquire the electrical signals (Leksrisawat et al. 2010; http://www.jove.com/index/details.stp?id=2323). Again, one could use the Backyard Brains equipment for a lower cost version to record electrical activity but requires one to make a suction electrode, design a dissecting microscope, and a simple manipulator in order to move the suction electrode.

The module can also be used as a template for examining other compounds of interest which may alter neural activity. Even simple changes in the ionic composition of the saline can be examined which would be inexpensive. For example, using a saline low in $Ca^{2+}$, by not adding $CaCl_2$, will cause the nerve to spontaneously produce action potentials and if one moves the joint while recording the activity there will be a difference from the activity in the initial normal saline. The low $Ca^{2+}$ saline can be exchanged back to normal saline and neural activity can be reassessed. This can be related to pathologies in mammals with hypocalcemia and heightened nerve excitability.

An instructor can go into a lot of depth with the biology of this module from how the sensory endings transform the mechanical energy to electrical through stretch activated channels and the type of channels to the production of action potentials and electrical conduction. Analogies to the muscle spindles in mammals can be discussed for monitoring proprioception.

The relationship with therapies for MS is an attractive topic for students and allows students to realize there is still a lot of experimentation with health care treatments outside of regulated clinical trials, such as the care of prescribing 4-AP in helping to manage MS. Promoting students to complete their own literature review allow students to realize the various methods researches use to try to understand different mechanisms on the topic of demyelinating diseases (see Rus et al. 2005; Coggan et al. 2015; Brugarolas et al. 2018).

The set of homework activities can be used as a pre- and post-test survey. One should not expect full answers on the pre-survey; however, after using the educational module one should expect full detailed answers on the post-survey using the same tool.

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