WPUNJ Animal Physiology Lab Manual

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Contents

The purpose of this Animal Physiology lab manual is to offer a guide for students to learn and apply physiological concepts through hands-on experiments and activities. Students will have the opportunity to work with living organisms along with dissecting preserved specimens. These living and preserved specimens are examples of arthropods, bony fish, amphibians, and mammals. Students will develop hypotheses, collect and analyze data to form scientific conclusions.

Various lab protocols were developed from iWorx Systems, Inc in order to utilize their software LabScribe to demonstrate physiological concepts, such as muscle physiology, neurophysiology, psychophysiology, fluid balance, and thermoregulation.

General Lab Rules

Prepare for each laboratory period by reading each exercise and becoming familiar with the principles and methods involved. By being familiar with the exercise you decrease the chances of an accident. Also, advance preparations allow you to use your time efficiently in the laboratory to complete the experiment within the given lab time.

Biology Laboratory Safety Rules

1. **No food or drink** is allowed in the lab **at any time**. Water bottles and coffee mugs can be left out in the hall.

2. **Always wear lab coat and if necessary glasses** when you work with chemicals, hot liquids, or other materials that could harm you.

3. **Wear nitrile gloves** while conducting molecular or chemistry in the laboratory.

4. **Always wear** closed toed shoes and long pants or a long skirt during labs to protect your legs and feet from spills.

5. **Always alert your lab instructor** to any spills on your skin or clothing. Hazardous chemical spills should be contained using the spill kit and disposed of in plastic bags.

6. **Never taste** or smell any chemicals.

7. **Tie or pin** long hair, scarves and headwear to avoid contact with flames or chemicals.

8. **Broken glassware** or sharp metal pieces should be placed in "sharps" boxes unless it is contaminated with body fluids or microorganisms. **Spills and breaks are part of working in a laboratory. The main thing to remember is NOT TO HURRY. Working in haste usually results in accidents or mistakes. Remember: if you are in a hurry or are distracted – you should not be working in the lab.**

If glass is broken, put on gloves and, using a paper towel, pick up large pieces of glass, placing them in the glass disposal container. Then contain the spill with paper towels, which should be placed in the biohazard waste bag for disposal, taking care to avoid injury by the smaller glass pieces. Using a clean paper towel, sweep the glass pieces into a dustpan and empty it into the glass disposal box. The floor or countertop and dustpan should be disinfected with 70% ethanol and a clean paper towel. If the culture is possibly pathogenic, follow the ethanol cleaning step with diluted Lysol and a clean paper towel.

9. Contaminated **sharps** should be placed in an appropriate labeled metal or glass container for sterilizing.

10. **Organic fluids** (e.g., ether, acetone, chloroform) or other volatile liquids should be used inside a fume hood.

11. **Chemical waste** should never be placed in a sink drain without permission. Please consult your TAs in all cases.

12. Smaller **solid waste** objects (pipette tips, etc.) should be placed in the small biohazard bags that are on each bench. Any trash that has not come into contact with the lab cultures (paper towel from hand washing, etc. can be thrown in the regular trashcans.

13. **Media with microorganisms** (i.e. bacteria, liquid or solid) should be placed in a red Biohazard bag or labeled autoclavable beakers for autoclaving sterilizing and disposal afterwards.

14. Begin lab work by disinfecting your work area. **Saturate the area with a disinfectant (70% ethanol) using a paper towel and allow the area to dry. Repeat this procedure after you have finished your work** to ensure that any material you have deposited on the work surface is properly disinfected. *Be sure to note if there are any open flames in the area before applying a flammable disinfectant to the bench.* Also spray a paper towel with 70% ethanol and wipe the barrels of micropipettors so they will be sanitized for the next user.

15. All culture material and chemicals should be properly labeled with your name, class, date, and experiment. **Labeling is critical to avoid improper use or disposal of material**. Label glass test tubes

with labeling tape – do not write with marker directly on glass tubes.

When disposing, remove tape from glassware that will be washed. It is not necessary to remove tape from items that will be disposed. Make a note of how stored samples are labeled. If using colored stickers for samples, putting a duplicate sticker (labeled identically), with additional descriptive annotations, in the laboratory notebook is convenient.

16. After the laboratory session, observe good hygiene by **washing your hands before leaving the laboratory**.

17. Guests and visitors in the laboratory are to be accompanied at all times by a registered student who is taking Biol 303. The safety of guests is the responsibility of the host student.

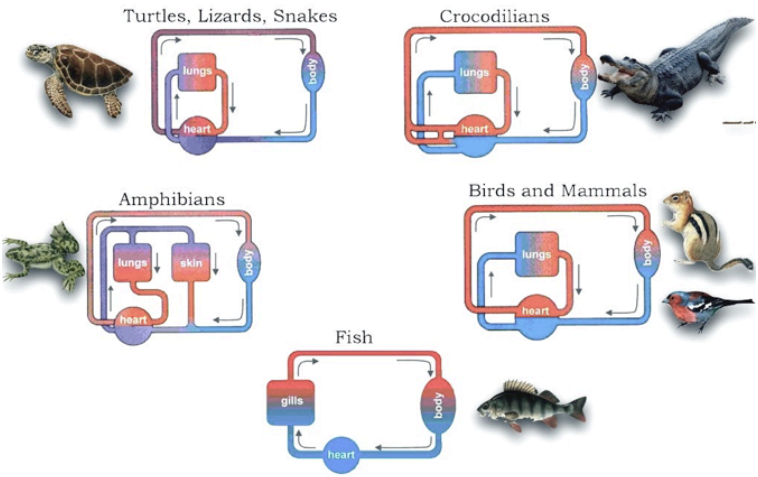
In the event of any accident or injury report immediately to available staff so that prompt and proper action can be taken. IN THE CASE OF BODILY INJURY PROCEED TO THE HEALTH CENTER OR CALL 911. THE RAVE GUARDIAN APP CAN ALSO BE USED IN EMERGENCIES. CLOSE THE LAB DOOR AND POST A SIGN ON THE DOOR WARNING OF THE SPILL OR BREAKAGE THAT HAS NOT BEEN CLEANED UP.

Comparative Physiology Oral Presentations

Background

Comparative physiology is a field within animal physiology that examines the similarities and differences in how living organisms function and have adapted to various environments, often focusing on how physiological systems have been altered in extreme environments. Understanding these adaptations from different model organisms leads to a better understanding of not only fundamental biological processes, but how they might be altered in a diseased state. Therefore, comparative physiology can result in significant medical breakthroughs to improve human quality of life.

Comparative physiology compares and contrasts biological systems that are required to sustain life, like the circulatory, nervous, respiratory, and endocrine systems, across different species. Comparative physiologists investigate how these systems have evolved and diversified in response to different environments and lifestyles. Typically, investigations explore how animals adapt to various environmental challenges like desiccation, temperature, salinity, and oxygen availability. Comparative physiology utilizes diverse organisms as models to understand human physiology and disease, as some species physiology in a given environment may be more similar to humans than traditional lab animals like mice. The field is known as being integrative, meaning that to fully understand the physiology, an understanding of multiple levels of biological organization is needed, this includes knowledge of molecules and cells to tissues, organs, and whole organisms.



Assignment

The goal of the assignment is to educate the class and gain additional exposure of different examples of how the physiological systems evolved across three different organisms adapting to a specific environment for survival as well as implications of this understanding for the benefit of humans, in a 15-minute oral presentation. First, however you will give a 2-minute elevator talk to the class describing the significance of your research that is geared towards a general audience (the mainstream public). See below for details on what is expected for the elevator talk and 15-minute oral presentation. Tips are given for giving an excellent oral presentation as well as a grading rubric at the end so that expectations are clear for the oral presentation.

How to make an *excellent* scientific oral presentation

When being graded on your oral presentation, you will be graded on the following two aspects: the content of the presentation and the delivery of the presentation. Although the content of the presentation will be more heavily weighted for grading purposes, the delivery of the presentation should not be overlooked as this can impede the understanding of the content of your presentation. In this lab you will have an opportunity to work on your oral presentation which you will present the next week.

Content of presentation

The following main areas must be covered in your presentation in this same following order, this is a standard convention when presenting scientific talks at conferences...

Elevator Talk

This is a 1-2 minute summary of what you did and what you found described in a way such that someone from the general public could understand what you are talking about. Include the importance of your analysis and why the general public should care about it. A very useful skill to have as a scientist, sounds easy but it actually takes some practice! You will give these before your oral presentation.

Oral Presentation

– *Introduction*

Starts off broad and then gets more specific. Attempts to capture the audience's attention. Contains general background information about the three organisms chosen for the comparative physiology analysis and a justification for picking them.

– *In-depth biological details of the physiological system you are comparing*

– A *discussion on how the physiological system is adapted for survival of the organism in a particular environment*

– A *comparison of the physiological system across the three species chosen*

– *Relevance for biological research and implications for humans of understanding how these physiological systems evolved in the different environments*

Delivery of presentation

E**www.elsevier.com**/connect/how-to-give-a-dynamic-scientific-presentation

Convey your ideas and enthusiasm – and avoid the pitfalls that put audiences to sleep

By Marilynn Larkin Posted on 4 August 2015

Share story:

Giving presentations is an important part of sharing your work and achieving recognition in the larger medical and scientific communities. The ability to do so effectively can contribute to career success.

However, instead of engaging audiences and conveying enthusiasm, many presentations fall flat. Pitfalls include overly complicated content, monotone delivery and focusing on what *you* want to say rather than what the audience is interested in hearing.

Effective presentations appeal to a wide range of audiences — those who work in your area of interest or in related fields, as well

as potential funders, the media and others who may find your work interesting or useful.

There are two major facets to a presentation: the content and how you present it. Let's face it, no matter how great the content, no one will get it if they stop paying attention. Here are some pointers on how to create clear, concise content for scientific presentations – and how to deliver your message in a dynamic way.

Presentation pointers: content

Here are five tips for developing effective content for your presentation:

1. **Know your audience.** Gear your presentation to the knowledge level and needs of the audience members. Are they colleagues? Researchers in a related field? Consumers who want to understand the value of your work for the clinic (for example, stem cell research that could open up a new avenue to treat a neurological disease)?

2. **Tell audience members up front why they should care and what's in it for them.** What problem will your work help solve? Is it a diagnostic test strategy that reduces false positives? A new technology that will help them to do their own work faster, better and less expensively? Will it help them get a new job or bring new skills to their present job?

3. **Convey your excitement.** Tell a brief anecdote or describe the "aha" moment that convinced you to get involved in your field of expertise. For example, Dr. Marius Stan, a physicist and chemist known to the wider world as the carwash owner on Breaking Bad, explained that mathematics has always been his passion, and the "explosion" of computer hardware and software early in his career drove his interest to computational science, which involves the use of mathematical models to solve scientific problems. Personalizing makes your work come alive and helps audience members relate to it on an emotional level.

4. **Tell your story.** A presentation is *your* story. It needs a beginning, a middle and an end. For example, you could begin with the problem you set out to solve. What did you discover by serendipity? What gap did you think your work could fill? For the middle, you could describe what you did, succinctly and logically, and ideally building to your most recent results. And the end could focus on where you are today and where you hope to go.

- *Start with context*. Cite research — by you and others — that brought you to this point. Where does your work fit within this context? What is unique about it? While presenting on organs-on-chips technology at a recent conference, Dr. Donald Ingber, Director of the Wyss Institute for Biologically Inspired Engineering at Harvard, described the pioneering work of others in the field, touched on its impact, then went on to show his unique contributions to the field. He did *not* present his work out of context, as though his group were the only one achieving results.
- *Frame the problem*: "We couldn't understand why our experiment wasn't working so we investigated further"; "We saw an opportunity to cut costs and speed things up."
- *Provide highlights* of what you did, tied to the audience's expertise and/or reasons for attending your presentation. Present the highlights in a logical order. Avoid going into excruciating detail. If people are interested in steps you don't cover, they'll ask and you can expand during the Q&A period. A meeting I covered on educational gaming gave presenters just 10 minutes each to talk about their work. Most used three to five slides, making sure to include a website address for more information on each slide. Because these speakers were well prepared, they were able to identify and communicate their key points in the short timeframe. They also made sure attendees who wanted more information would be able to find it easily on their websites. So don't get bogged down in details — the *what* is often more important than the *how*.

- *Conclude by summing up key points* and acknowledging collaborators and mentors. Give a peek into your next steps, especially if you're interested in recruiting partners. Include your contact details and Twitter handle.

5. **Keep it simple**. Every field has its jargon and acronyms, and science and medicine are no exceptions. However, you don't want audience members to get stuck on a particular term and lose the thread of your talk. Even your fellow scientists will appreciate brief definitions and explanations of terminology and processes, especially if you're working in a field like microfluidics, which includes collaborators in diverse disciplines, such as engineering, biomedical research and computational biology.

I've interviewed Nobel laureates who know how to have a conversation about their work that most anyone can understand – even if it involves complex areas such as brain chemistry or genomics. That's because they've distilled their work to its essence, and can then talk about it at the most basic level as well as the most complicated. Regardless of the level of your talk, the goal should be to communicate, not obfuscate.

Presentation pointers: you

Here are 10 tips to help you present your scientific work and leave the audience wanting more.

1. **Set the stage.** Get your equipment ready and run through your slides if possible (use the "speaker ready" room if one is available). If you've never been in the venue, try getting there early and walk the room. Make sure you have water available.

2. **Get ready to perform.** Every presentation is a performance. The most important part is to know your lines and subject. Some people advocate memorizing your presentation, but if you do so, you can end up sounding stilted or getting derailed by an

interruption. When you practice, focus on the key points you want to make (note them down if it helps) and improvise different ways of communicating them.

Related resource

It's well known that a majority of people fear public speaking — and even those who enjoy it may get stage fright. Fear of public speaking will diminish with experience. I've been presenting and performing for many years but still get stage fright. Try these strategies to manage the fear:

- *Breathe* slowly and deeply for a few minutes before your talk.
- *Visualize* yourself giving a relaxed talk to a receptive audience. This works best if you can close your eyes for a few minutes. If you're sitting in the audience waiting to be introduced and can't close your eyes, look up at the ceiling and try visualizing that way.
- *Do* affirmations. Tell yourself you are relaxed, confident — whatever works for you. Whether affirmations are effective is a matter of debate, but you won't know unless you try.
- *Assume* one or more "power poses," developed by social psychologist and dancer Dr. Amy Cuddy of the Harvard Business School, before giving your presentation. She demonstrates them in this TED talk. Power poses are part of the emerging field of embodiment research (see a comprehensive collection of articles related to this research in the journal *Frontiers in Psychology*). Research on power poses has yielded mixed results to date, but they're worth a try.

3. **Stride up to the podium.** Seeing you walk energetically energizes the audience. They expect you to engage them and you have their attention.

4. **Stand tall and keep your chest lifted**. It's more difficult to breathe and speak when your shoulders are rolled forward and your chest caves in. Standing tall is also a way of conveying authority. If you're presenting from a sitting position, sit up in your seat, keep your arms relaxed and away from your sides (i.e., don't box yourself in by clasping your arms or clasping your hands in your lap).

5. **Smile.** Not only will you appear more relaxed if you smile, but research has shown that smiling — even when forced — reduces stress. Plus the audience enjoys watching and listening to someone who's smiling rather than being stern or overly serious, especially if your topic is complicated.

One of the most enjoyable presentations I've covered was on animal versus human cognition. It dealt with the evolution and activation of different parts of the brain. By inserting anecdotes in with complex didactic information, presenter Dr. Onur Güntürkün, Professor of Biological Psychology at the Ruhr-Universität Bochum in Germany, made the topic accessible and compelling.

6. **Speak up.** The audience came to your talk so they really do want to hear what you have to say. If a microphone is available, use it. I've seen countless presenters stand in front of a microphone yet somehow manage not to talk into it. Talk from your diaphragm, not your throat, to give your voice authority and resonance.

7. **Take your time.** A moment or two of silence as you gather your thoughts or move to a new topic can actually make the audience pay attention. Don't feel you have to talk continuously, and avoid filler phrases, such as "you know."

8. **Talk to the audience, not the screen.** Making eye contact with one or more friendly faces can relax you and help you connect to the audience. It will also prevent you from reading your slides, which you don't want to do unless absolutely necessary (for example, if you forget the statistics supporting a particular point).

9. **Stick to your time frame.** We've all done it, but it's not fun to have to cycle rapidly through your last 10 slides because the moderator has given you a two-minute warning and you're nowhere near the end. Try to pace yourself. When preparing your slides and

practicing (i.e., rehearsing for your performance), make a note on the slide you think you should be discussing when you're about midway through your talk. This gives you a benchmark and lets you know if you need to speed up or slow down the rest of the presentation.

10. **Don't drift off at the end.** I've seen people read their summary slide, then nod and walk away. Instead, say "That concludes my presentation. Thank you for your attention." If appropriate, ask if there are any questions or tell the audience they will have an opportunity to ask questions later.

Six dos and don'ts of PowerPoint slides

1. **Less is more.** Although there are no "rules," I've found that 20-25 slides work well for a one hour presentation. You'll have a better idea what works for you if you time yourself during a practice session.

2. **Create sections.** Use a title slide to start a new section or change the subject. This will also help you organize your presentation and make sure it flows logically.

3. **Avoid clutter.** Stick to three to five bullet points per slide *at most*. Bullet points should contain key words — not complete sentences. For examples of what *not* to do, see this recent editorial in the *Washington Post*, which urges a ban on PowerPoint presentations.

4. **Make it readable**. Rule of thumb for fonts: 28-40 point for headlines; 18-28 for text; 12-14 for references. Use *sans serif* fonts, and make sure you have a strong contrast between the background and text (e.g., black or dark blue text on a white background; white text on a blue background). Don't use ALL CAPS; underscore a point by putting it in italics or bold (underlining can make the text more difficult to read).

5. **Use visuals.** In a recent talk, presenters explained why biological image processing and analysis is a hot field in laboratory

R&D. The reason is simple: you can tell a lot more about cells with an image versus a cell count. The same is true of your presentation: a single image of something particularly relevant to your work is more engaging and has the potential to convey more information than words.

That said, it's important to keep the visual simple — an image of a single cell or pathway, for example. If you use graphs to show comparisons or results, indicate what the axes represent and which variables (ideally, not more than two or three) you're displaying.

Generally, steer clear of videos. One of the few effective videos I've seen was of a Caledonian crow creating a tool to obtain food, which Dr. Güntürkün included in the presentation referred to above. Videos of in vitro experiments and imaging results rarely help support a point because the low resolution makes everything look grainy.

6. **Check your spelling**. Nothing takes away from credibility like misspelled words, especially if they're up on large screen for a minute or more — or worse, repeated throughout your presentation. After you use spell check, proof your presentation yourself. Let a day go by if possible; it's easier to pick up errors after a break.

Lab 1: Oral presentation grading rubric

Grading Rubric for oral presentation (25 points)

Name of student:_____Date:_____

		Comments	Points accrued	Maximum points possible
Content of presentation – contains background information about the organisms chosen, goes into detail of a physiological system with an explanation of how this system functions and has adapted to a given environment, then links this to its survival. Compares the physiological system across three organisms, discusses the importance of the analysis for biological research and how it can potentially benefit humans.				10
Delivery of presentation – a good amount of eye contact, body language is appropriate, loud and clear voice, refers to slides or poster when discussing relevant points, good transitions between slides so that the presentation flows well. The presentation is no more than 15 minutes.				5
Visual appeal of presentation – the slides/poster are well balanced and visually appealing, there is enough contrast and the text is easy to read, the figures are formatted so they are easy to read. There are headings to identify the separate parts of the presentation.				2.5
Elevator talk – the talk is gauged for a general audience, it is succinct, clear, well-rehearsed, and provides an overview an importance of the comparative analysis. The talk lasts around no more than 2 minutes.				2.5

Literature cited – references properly formatted in a consistent scientific journal style, this is presented at the end of the talk as full references.				2.5
Answering questions – is able to give some sort of educated answer to the questions asked, which reflects additional reading of previous literature, questions are asked when others are presenting.				2.5

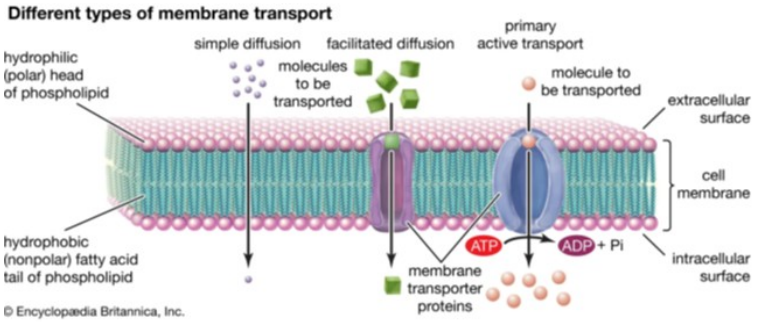
Lab 1: Membrane Physiology

Step 1: Read the background information before starting the first activity.

Exercise Overview:

The molecular composition of the plasma membrane allows it to be selective about what passes through it. It allows nutrients and appropriate amounts of ions to enter the cell and keep out undesirable substances. For that reason, we say that the plasma membrane is **selectively permeable**. Valuable cell proteins and other substances are kept within the cell, and metabolic wastes pass to the exterior. Transport through the plasma membrane can occur in two basic ways: either passively or actively. In **passive processes**, the transport process is driven by concentration or pressure differences (*gradients*) between the interior and exterior of the cell. In **active processes**, the cell provides energy (ATP) to power the transport. Two key passive processes of membrane transport are **diffusion** and **filtration**. Diffusion is an important transport process for every cell in the body. **Simple diffusion** occurs without the assistance of membrane protein, and **facilitated diffusion** requires a membrane-bound carrier protein that assists in the transport. In both simple and facilitated diffusion, the substance being transported moves *with* (or *along* or *down*) the *concentration gradient* of the solute (from a region of its higher concentration lo a region of its lower concentration). The process does not require energy from the cell. Instead, energy in the form of **kinetic energy** comes from the constant motion of the molecules. The movement of solutes continues until the solutes are evenly dispersed throughout the solution. At this point, the solution has reached **equilibrium**.

A special type of diffusion across a membrane is **osmosis**. In osmosis, water moves with its concentration gradient. From a higher concentration of water to a lower concentration of water. It moves in response to a higher concentration of solutes on the other side of a membrane. In the body, the other key passive process, **filtration**, usually occur only across capillary walls. Filtration depends upon a *pressure gradient* as its driving force. It is not a selective process. It is dependent upon the size of the pores in the filter. The two key active processes (recall that active processes require energy) are **active transport** and **vesicular transport**. Like facilitated diffusion, active transport uses a membrane bound carrier protein. Active transport differs from facilitated diffusion because the solutes move *against* their concentration gradient and because ATP is used to power the transport. Vesicular transport includes phagocytosis, endocytosis, pinocytosis, and exocytosis. These processes are not covered in this exercise. The activities in this exercise will explore the cell transport mechanism individually.



Step 2: complete the **five** activities of the Cell Transport Mechanisms and Permeability Exercise located in the PhysioEx software program that can be found on the desktop of the lab computer. Note: Make sure to follow the activity step by step.

Make sure to take the pre-lab and post-lab quizzes before moving on to the experimental simulations.

To summarize your results, fill out the corresponding tables in your lab report sheet for the first **four** activities **as you complete the activity** that focus on each of the key concepts related to membrane physiology.

Note: You will obtain the values for the tables once you hit the record data button after running an experimental simulation at the bottom of the screen.

You can skip the review sheet questions as some of these are located on your lab report sheet instead.

Step 3: be sure to answer all of the questions in the lab report sheet and to ask any questions you may have stemming from the activities before leaving the lab.

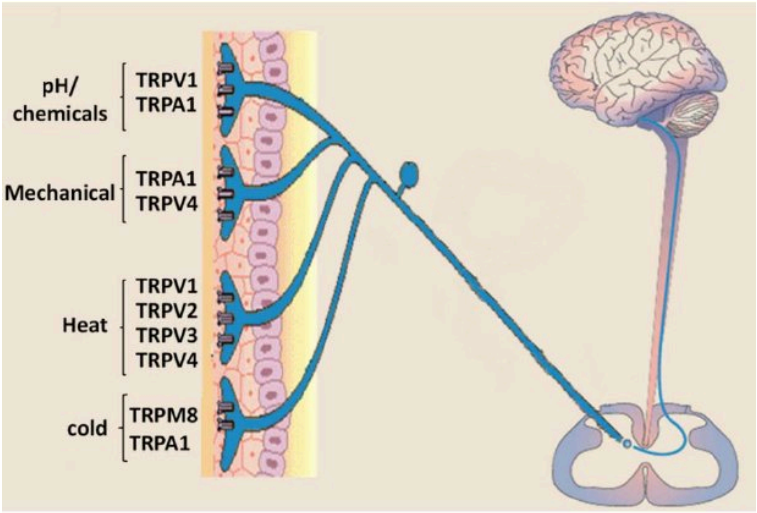
Note: You can save the results from each activity as a PDF report if you would like to study from it later on.

Lab 2: Action Potential

Step 1: Read the background information before starting the first activity.

Exercise Overview:

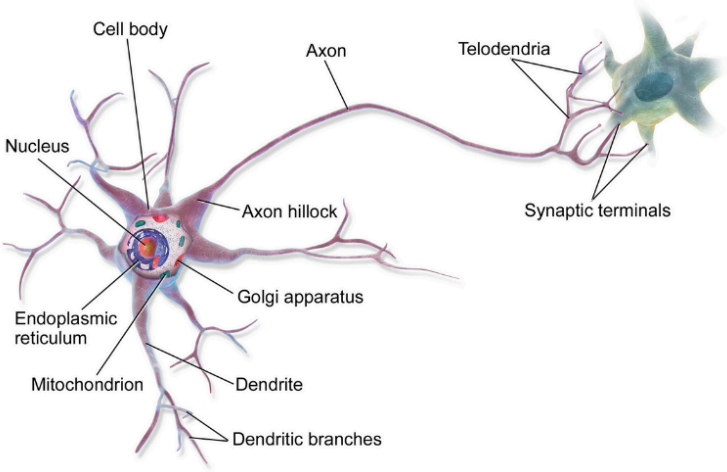
The nervous system contains two general types of cells: **neurons** and neuroglia (or glial cells). This exercise focuses on neurons. Neurons respond to their local environment by generating an electrical signal. For example, sensory neurons in the nose generate a signal (called a **receptor potential**) when odor molecules interact with receptor proteins on the membrane of these olfactory sensory neurons. Thus, sensory neurons can respond directly to sensory stimuli. The receptor potential can trigger another electrical signal (called an **action potential**), which travels along the membrane of the sensory neuron's axon to the brain – you could say that the action potential is conducted to the brain.



The action potential causes the release of **chemical neurotransmitters** onto neurons in olfactory regions of the brain. These chemical neurotransmitters bind to receptor proteins on the membrane of these brain **interneurons**. In general, interneurons respond to chemical neurotransmitters released by other neurons. In the nose the odor molecules are sensed by sensory neurons. In the brain the odor is perceived by the activity of interneurons responding to neurotransmitters. Any resulting action or behavior is caused by the subsequent activity of **motor neurons**, which can stimulate muscles to contract (see Exercise 2).

In general, each neuron has three functional regions for signal transmission: a receiving region, a conducting region, and an output region, or secretory region. Sensory neurons often have a receptive ending specialized to detect a specific sensory stimulus, such as odor, light, sound, or touch. The **cell body** and **dendrites** of interneurons receive stimulation by neurotransmitters at structures called **chemical synapses** and produce **synaptic potentials**. The

conducting region is usually an **axon**, which ends in an output region (the axon terminal) where neurotransmitter is released.

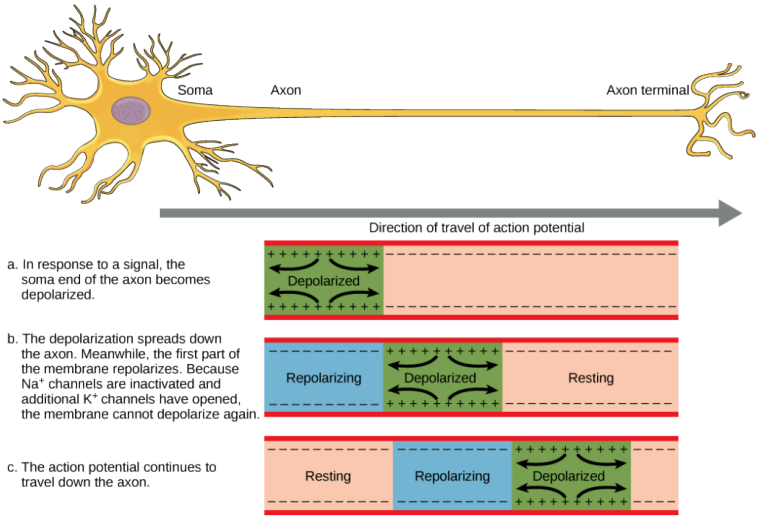


Although the neuron is a single cell surrounded by a continuous plasma membrane, each region contains distinct membrane proteins that provide the basis for the functional differences. Thus, the receiving end has receptor proteins and proteins that generate the receptor potential, the conducting region has proteins that generate and conduct action potentials, and the output region has proteins to package and release neurotransmitters. Membrane proteins are found throughout the neuronal membrane-many of these proteins transport ions (see Exercise I).

The signal generated and conducted by neurons are electrical. In ordinary household devices, electric current is carried by electrons. In biological systems, currents are carried by positively or negatively charged **ions**. Like charges repel each other and opposite charges attract. In general, ions cannot easily pass through the lipid bilayer of the plasma membrane and must pass through **ion channels** formed by integral membrane proteins. Some channels are usually open (leak channels), and others are gated, meaning that

the channel can be in an open or closed configuration. Channels can also be selective for which ions are allowed to pass. For example, sodium channels are mostly permeable to sodium ions when open, and potassium channels are mostly permeable to potassium ions when open. The term **conductance** is often used to describe **permeability.** ln general, ions will flow through an open channel from a region of higher concentration to a region of lower concentration (see Exercise 1). In this exercise you will explore some of these characteristics applied to neurons.

Although it is possible to measure the ionic currents through the membrane (even the currents passing through single ion channels), it is more common to measure the potential difference. or voltage, across the membrane. This membrane voltage is usually called the **membrane potential**, and the units are in **millivolts (mV)**. One can think of the membrane as a battery, a device that separates and stores charge. A typical household battery has a positive and a negative pole so that when it is connected, for example through a lightbulb in a flashlight, current flows through the bulb. Similarly, the plasma membrane can store charge and has a relatively positive side and a relatively negative side. Thus, the membrane is said to be **polarized**. When these two sides (intracellular and extracellular) are connected through open ion channels. current in the form of ions can flow in or out across the membrane and thus change the membrane voltage.



Step 2: in the PhysioEx 10.0 software on the desktop of the computer, open it, and click on **Exercise 3: Neurophysiology of Nerve Impulses.** Start with the first activity and be sure to take the pre and post-lab quizzes.

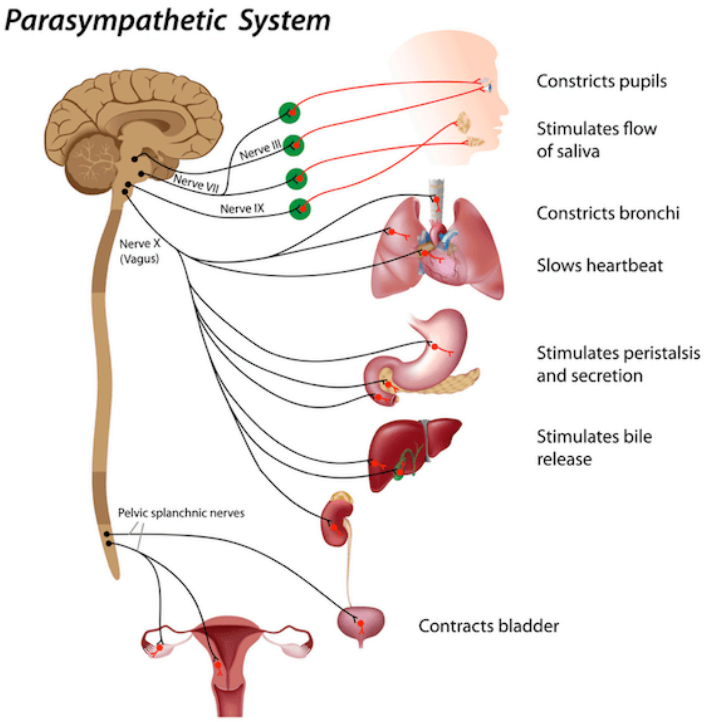
You can skip the second activity and then complete activities 3-8 of **Exercise 3: Neurophysiology of Nerve Impulses** from the PhysioEx 10.0 software on the desktop of the computer.

Step 3: record the data obtained from the activities in the tables in your lab report document that will be graded for accuracy **as you complete the activities**. In addition, be sure to complete the questions at the end of the lab report and to ask any questions you may have about these during the lab session as these will be graded as well.

Lab 3: Heart Rate, Blood Pressure, and Vagal Tone: The relationship between personality and vagal Tone

Background

Vagal tone, a measure of parasympathetic nervous system activity through the vagus nerve, is thought to be linked to personality traits and emotional regulation.



Personality is defined as a set of behavioral traits that tend to be correlated with one another and for humans, define their character. For animals, some examples of personality traits include individuals who tend to be more bold, risk-prone, and impulsive, while others within the same species or a population of species may tend to be, in contrast, more shy, risk-averse, and have higher levels of self-control.

Previous research has shown that individuals with higher vagal tone tend to exhibit greater resilience, better emotion regulation, and stronger social bonds, while those with lower vagal tone may be more susceptible to anxiety, depression, and difficulties with emotional control. Each of these traits can prove to be beneficial

depending upon the environment you are living in. For example, if food is scarce or there is a stressful environment in general, then it is beneficial to be more impulsive and have less self-control, as well as being more cautious with higher levels of anxiety.

Exercise 1: Personality and Vagal Tone

Aim: To test a hypothesis that persons with high perceived shyness and behavioral inhibition have lower Vagal tone than persons with low perceived shyness. Vagal tone is measured as the difference between the maximum and minimum heart rates of the subject during normal breathing.

Start the Software

1. Click on the LabScribe shortcut on the computer's desktop to open the program. Note: when you open the LabScribe software, you may have to select iwx214 (first choice) for the hardware.
2. On the Main window, pull down the Settings menu and select Personality-Vagal Tone under the Human Psychophysiology menu.
3. After a short time, LabScribe will appear on the computer screen as configured by the Personality- Vagal Tone settings.

Set up

1. Wrap the elastic belt of the respiration monitor around the subject's chest at a level that is below the sternum. Place the sensor inside the belt so that the sensor is in the center of the chest at a level that is even with the subject's elbows.

2. Wrap the pulse plethysmograph around the subject's right hand middle finger.

Procedure

1. Instruct the subject to sit quietly and breathe normally before and during the recordings to prevent the creation of motion artifacts. The subject should sit erect so that the muscles involved in pulmonary ventilation are able to move without restriction.
2. Type Normal Breathing <Subject's Name> in the Mark box to the right of the Mark button.
3. Click Record. Press the Enter key on the keyboard. Click AutoScale for all four channels. Record the subject's breathing and heart rates for at least one minute.
4. Click Stop to halt recording.
5. Select Save in the File menu.

Data Analysis

1. Scroll through the data file and find the recording of the subject's heart and breathing rates while breathing normally. Note: when you open the LabScribe software, you may have to select iwx214 (first choice) for the hardware.
2. Use the Display Time of the Main window to show an artifact-free section of data containing five breath cycles in the Main window. This section of data can also be selected by:

- Placing the cursors on either side of the section of data needed.
- Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window.

3. Click on the Analysis window icon in the toolbar (Figure HP-5-L3) or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.

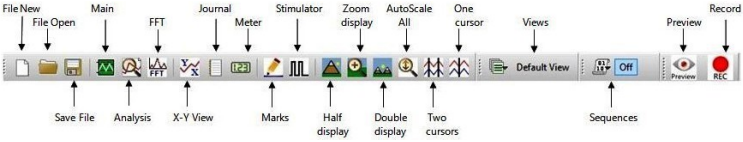


Figure HP-5-L3: LabScribe toolbar

4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions that are listed should include Value1, Value2, V2-V1, T2- T1, Max, Min, and Mean. The values for these parameters from each channel are seen in the table across the top margin of each channel.

5. Once the cursors are placed in the correct positions for determining the maximum, minimum, and mean heart rates, these rates can be recorded in the on-line notebook of LabScribe by typing the names and values directly into the Journal.

6. To use the Journal: The functions in the channel menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:

- Place the cursors at the locations described below (step 4) to measure the Heart and Ventilation Rates.
- Transfer the names of the mathematical functions used to determine the heart and/or ventilation rates to the Journal by right clicking in the correct channel from which you are gathering the data and selecting the 'Add Title to Journal' function in the menu. This will provide the channel titles to correspond to data.

*NOTE: If you would like the unit of measure to be added to your

table, you must click on the Add function Button and select 'unit' for it to show up.

- Transfer the values for specific channels, such as heart and ventilation rates to the Journal by right clicking and selecting the 'Add Ch. Data to Journal' function in the menu. This will copy the data for specific channels to your journal.
- To copy all data gathered, right click and select 'Add all Ch. Data to Journal' function in the menu. This will copy all data from all channels to the journal.

**NOTE: The data copied and pasted thus far is under the 'table' icon and is not in the final journal format. To place all data from the table into the journal, select all data by right clicking and coping selection, click on Editor Button (next to table button) and paste in the document.

7. On the Respiration channel, click and drag one cursor to the beginning of the first breath cycle displayed in the Analysis window (Figure HP-5-L1). Drag the other cursor to the beginning of the second breath cycle and measure the following:

a. Maximum Heart Rate. The value for Max on the Heart Rate channel is the subject's maximum heart rate during the first breath cycle.

b. Minimum Heart Rate. The value for Min on the Heart Rate channel is the subject's minimum heart rate during the first breath cycle.

c. Mean Heart Rate. The value for Mean on the Heart Rate channel is the subject's mean heart rate during the first breath cycle.

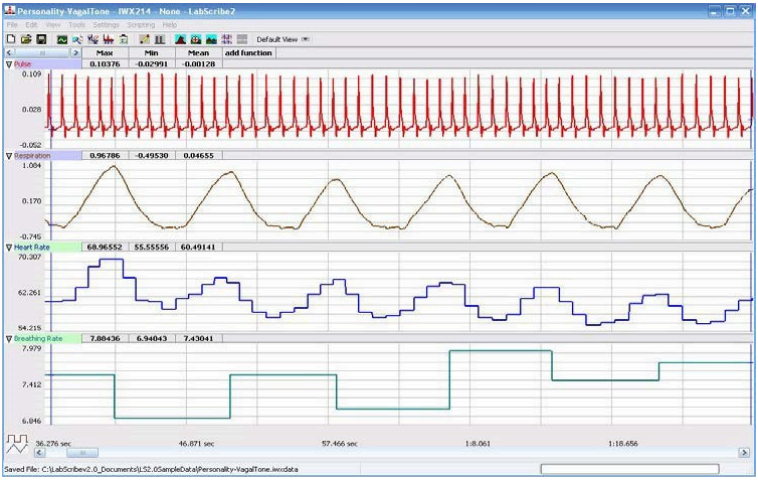


Figure HP-5-L1: The pulse wave, respiration, heart rate, and breathing rate recordings of a subject using abdominal breathing while at rest are displayed, from top to bottom, in the Analysis window. On the respiration channel, inhalation is displayed as an upsweep. Notice that the heart rate goes up during inhalation.

8. On the Respiration channel, leave one cursor at the beginning of the second breath cycle. Click and drag the other cursor to the beginning of the third breath cycle displayed in the Analysis window.

9. Repeat Steps 7 and 8 for the second breath cycle.

10. Move the cursors to the beginning and end of each of the three remaining breath cycles and repeat Steps 7 and 8 for each breath cycle

11. Determine the means of the maximum and the minimum heart rates for the five breaths. Enter these values in Table HP-5-L1.

Table HP-5-L1: Heart Rate Variation during Normal Breathing

	Heart Rate (BPM)			Breath Rate (BrPM)
Subject	Max	Min	Δ	
Breath 1				
Breath 2				
Breath 3				
Breath 4				
Breath 5				
Mean				

12. Determine the difference (Δ) between the mean maximum and mean minimum heart rates and enter this value in Table HP-5-L1. This difference, which will be used to measure Vagal tone, is also known as the respiratory sinus arrhythmia (RSA) prominence.

13. On the Respiration channel, click and drag one cursor to the beginning of the first breath cycle. Click and drag the other cursor to the end of the fifth breath cycle displayed in the Analysis window and measure the Mean Breath Rate. The value for Mean on the Breath Rate channel is the subject's mean breath rate over the five consecutive breath cycles.

14. Record the value for this rate in the Journal using one of the techniques described in Exercise 1, and in the data table.

15. Have the subject answer the questions in the shyness/ behavioral inhibition questionnaire in Table HP-5-L2.

Step 2: Enter the data you have collected into a provided excel spreadsheet that is located on the computer, at the front of the room, to compile the class data.

Use the SPSS software on the lab computer and obtain the completed datasheet and run a spearman rank correlation analysis between personality score and the mean difference in the min and max heart rate (Vagal tone), as well as the personality score, and the breath rate. Make two different X,Y correlation plots with the r value and and p-value being presented on the plot from your spearman rank correlation.

Step 3: use the following questions below and the analysis above to generate result statements and a corresponding concluding paragraph that interprets these results based on the class results obtained. These will be turned in for grading as a lab report.

Questions

1. What is the value for the Vagal tone of the subject? What percentage of the mean minimum heart rate is the value for the Vagal tone?
2. How does the Vagal tone of this subject compare to the subject's score on the shyness/behavioral inhibition questionnaire?
3. How does the Vagal tone of this subject compare to those of other subjects? When the Vagal tones and shyness scores of all the subjects are compared, is there a correlation between shyness/behavioral inhibition and Vagal tone?
4. Does any other factor besides shyness or inhibition affect Vagal tone?

HP-5-L2: Shyness/Behavioral Inhibition Questionnaire*

Question	
How often do you experience awkwardness or discomfort in the following situations?	Score (0-4)
1. At a party	
2. On a dinner date	
3. In a class discussion when expected to contribute	
4. In an uncrowded elevator	
5. When introduced to someone	
6. In a study group	
7. When asked to introduce yourself in class	
8. When asking someone out for coffee (tea, soda, etc.)	
9. When asking for notes from a classmate after an absence	
10. When speaking to a professor	
How often do you have difficult actually doing (taking action on) the following?	Score (0-4)
11. Making and sustaining eye contract in a conversation.	
12. Initiating a conversation with persons you do not know well.	
13. Disagreeing with someone.	

14. Asking a question in class.	
15. Making small talk.	
16. Asking directions or help.	
17. Calling people on the phone to invite them over.	
18. Giving aide or attention to someone in distress.	
19. Seeking information from others about class assignments	
20. Telling someone he or she is bothering you.	
TOTAL	
*Ratings: 0 = never, 1 = rarely, 2 = occasionally, 3 = often, 4 = almost always. The total score is the sum of the ratings for all 20 questions.	

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Lab 4: Chemo-sensory Integration

Sensory physiology is the branch of neuroscience that studies how our bodies detect, process, and interpret sensory information from the environment.

Key sensory physiology concepts:

Sensory Receptors: Specialized cells or tissues that detect specific stimuli, such as light, sound, chemicals, or pressure.

Transduction: The process by which sensory receptors convert stimuli into electrical signals that can be transmitted to the nervous system.

Sensory Pathways: The neural circuits that carry sensory information from receptors to the brain.

Perception: The conscious experience resulting from the interpretation of sensory signals in the brain.

Examples of Sensory Physiology:

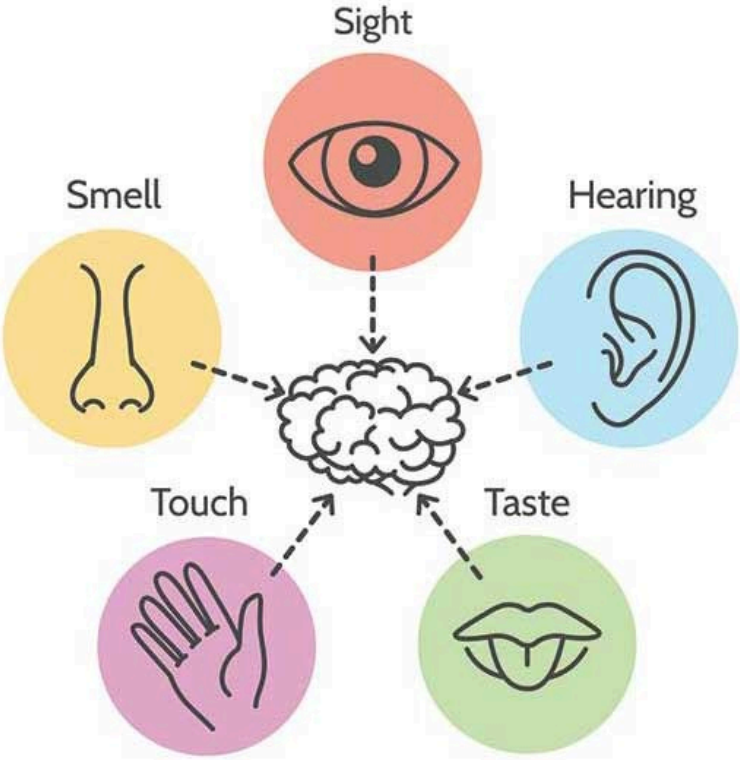
Vision: How the eye detects light and sends information to the brain to create images.

Hearing: How the ear converts sound waves into electrical signals that are interpreted as sound.

Smell: How the nose detects odor molecules and sends signals to the brain for identification.

Taste: How the tongue detects chemicals in food and sends information to the brain for flavor perception.

Touch: How the skin detects pressure, temperature, and pain and transmits these signals to the brain.



Sensory physiology plays a crucial role in understanding how we interact with our environment and perceive the world around us. It is also essential for diagnosing and treating sensory disorders, such as vision loss, hearing impairment, and loss of smell or taste. Additionally, sensory physiology has implications for fields like robotics, artificial intelligence, and human-computer interaction.

Chemo-sensory processes specifically involve taste and smell. In insects, olfaction enables the organism to recognize volatile cues that facilitate the detection of food, predators, and mates. In

contrast, the sense of taste commonly allows the discrimination of soluble stimulants that elicit feeding behaviors and can also initiate innate sexual and reproductive responses. The sensitivity to these stimuli in honey bees exceeds even a dog's smelling ability, which is far greater than humans. Therefore, the honey bee is an ideal model organism for testing the preferences of food rewards and possibly addiction.

Recent research has shown that honey bees may become addicted to alcohol, which is naturally produced from the fermentation of nectar as it sits in flowers waiting to be consumed. To demonstrate whether honey bees can be used as a model organism for studying alcohol addiction, three main questions arise: 1) Can alcohol have a hedonic value that is sought after by bees?, 2) Is the acute effect of alcohol on bees similar to that on mammals?, and 3) Do chronic doses of alcohol induce similar effects as in mammals, i.e. signs of addiction?

To answer questions 1 and 2, you will conduct what is known as a Proboscis Extension Response (PER) assay, which is a powerful behavioral standardized assay for honey bees to determine their preference or appetite for food. This is measured by touching a droplet of food stimulus (usually sucrose solution) to the harnessed honey bee's antenna and then determining whether or not the bee sticks out its proboscis in an attempt to feed on that solution, without letting the honey bee feed on the solution. A series of solutions can be tested in ascending order to determine their preference.

Half of the class will be testing honey bees using the PER assay that were previously fed 4 μL of 50% sucrose solution without any alcohol (negative control). The other half of the class will be testing honey bees that were fed 4 uL of 50% sucrose solution with the addition of 2.5% ethanol, their preferred level of alcohol in the food that they consume.

To prevent any potential observation bias the feeding treatment will be blind to the observer and therefore, you will only know the group letter you are working with, either A or B.

Materials needed per group (4 groups total):

20 µL micropipette

20 uL micropipette tips

1.5 mL microcentrifuge tubes in a rack with 1 mL of the following solutions 30% sucrose solution with 0%, 1%, 5%, 10%, and 20% and 50% ethanol (molecular grade), the water should be distilled water. An additional 1.5 mL microcentrifuge tube with 1 mL of distilled water.

Color of Tube	Solution
Clear	50% sucrose in distilled water
Clear	50% sucrose in 2.5% ethanol
Purple	30% sucrose in distilled water (0% ethanol)
Orange	30% sucrose in 1% ethanol
Green	30% sucrose in 5% ethanol
Pink	30% sucrose in 10% ethanol
Yellow	30% sucrose in 20% ethanol
Blue	30% sucrose in 50% ethanol

Supplies for collecting forager bees

4 Peg trays

40 cut plastic drinking straws

40 pieces of duct tape 1 mm in width, cut on a glass cutting board using a razor blade

40 forager honey bees

1 Bucket of crushed ice

40 glass vials (20 mL)

50% sucrose solution (1 mL), 50% sucrose solution w/ 2.5% ethanol (molecular grade) (1mL)

20 uL micropipette, 20 uL micropipette tips

Safety note: you will be working with live honey bees that can sting, if you already know you are deathly allergic to bee stings then let the instructor know.

If one escapes from the harness then just let it fly towards the

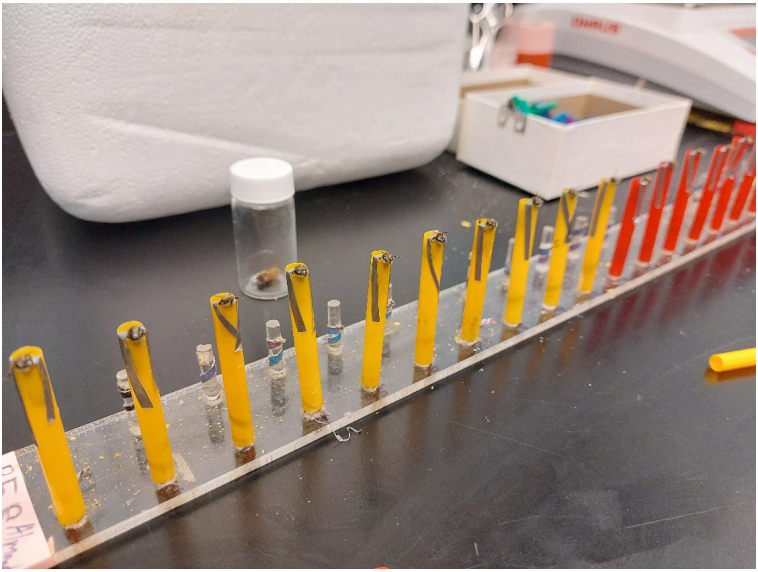
window or the lights above, the instructor will catch it with a glass vial.

Procedure:

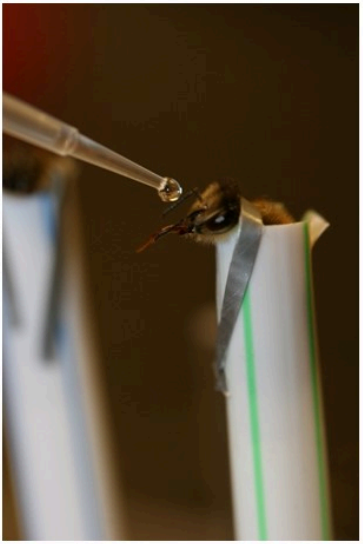
Step 1: take note of the treatment group (either A or B) of honey bees you are working on your lab report datasheet, which should be labeled on the peg tray.

Step 2: Start with the distilled water and touch the honey bee's antennae with a droplet of liquid using the 20 µL micropipette (see below). Do this for every bee on the peg tray in order and record whether the proboscis is out for each bee. Then repeat this procedure in ascending order of alcohol concentration (0%, 1%, 5%, 10%, 20%, 50%) in 30% sucrose solution, but touch the bee's antennae with water in between each round using the 20 µL micropipette. **Make sure you do not allow the bee to feed on the sucrose solution!**

Have another student in the group be an observer, while the other will be the data recorder to record whether or not the honey bee sticks out its proboscis. Rotate the jobs each round per concentration of alcohol.



Harness honey bees in cut plastic drinking straws that are ready for PER testing.



Proboscis Extension Response (PER) assay

An example of the PER assay, where the antennae of the honey

bee are touched with a solution, and the proboscis sticks out in an attempt to feed on this solution.

Step 3: compile your data and enter it into the class excel spreadsheet at the front of the room.

Step 4: make a line graph using excel of the class data comparing the two honey bee treatment groups (control versus alcohol treated). Put this in your lab report and make sure your axes are labeled.

Step 5: sum up the number of proboscis responses just for the treatments with sucrose solution and alcohol (not the water only treatments) across all of the concentrations for each group of honey bees. Conduct a chi-square goodness of fit test using a calculator online. Obtain your test statistic and p-value and write a results statement underneath your graph in your lab report and make a figure caption for your graph.

Step 6: write a concluding paragraph underneath your figure legend in your lab report based on the results obtained that relate your results to your initial hypothesis and study aim.

Lab 5: Introduction to Organismal Systems

Disclaimer: This lab will involve the dissection of once live organisms, and at times, this practice has been controversial, but these organisms have been humanely euthanized and preserved with educational purposes in mind. In order to facilitate the accurate learning of anatomical structures, it is necessary to observe and dissect an actual specimen as opposed to performing a virtual dissection. If, at any time, you are not feeling well, you can always step out of the lab and take a break. If you are adverse to doing the cutting, then pair up with someone who doesn't mind, you will however, still be responsible for observing and participating in the lab activity.

Purpose: In this lab, you will dissect an earthworm, yellow perch bony fish, and a rat in order to observe their external and internal structures. After identifying the external and internal structures, compare them across the organisms and consider how they have been adapted of a "design" that is particularly suited for the habitat that they live in.

Think about the following questions as you conduct your dissections today. How are the organismal structures similar? How are they different? How are the structures linked to their function? How have they been altered through evolutionary history? Which structures are similar to humans? How are they different?

Directions: Once you have identified all of the external structures for each organism have your instructor quiz you on them, then move onto the internal structures and repeat the same process before moving onto the next organismal dissection. Make drawings of the external and internal anatomy, with labels, of each organism, to be graded as your lab report.

Dissection of Earthworm (annelid)

Background: Among the most familiar invertebrate animals are the earthworms, members of the phylum **Annelida.** The word annelida means "ringed" and refers to a series of rings or segments that make up the bodies of the members of this phylum. Internally, **septa**, or dividing walls, are located between the segments. There may be more than 100 segments in an adult worm. http://en.wikipedia.org/wiki/Earthworm

Digestive System: The earthworm takes in a mixture of soil and organic matter through its mouth, which is the beginning of the digestive tract. The mixture enters the **pharynx**, which is located in segments 1–6. The **esophagus**, in segments 6–13, acts as a passageway between the pharynx and the crop. The **crop** stores food temporarily. The mixture that the earthworm ingests is ground up in the **gizzard**. In the **intestine**, which extends over two-thirds of the body length, digestion and absorption take place. Soil particles and undigested organic matter pass out of the worm through the **rectum** and **anus**.

Reproductive system: The **clitellum** is a swelling of the body found in sexually mature worms and is active in the formation of an egg capsule, or **cocoon**. **Eggs** are produced in the **ovaries** and pass out of the body through **female genital pores**. **Sperm** are produced in the **testes** and pass out through tiny **male genital pores**. During mating, sperm from one worm travel along the sperm grooves to the **seminal receptacles** of another worm. Fertilization of the eggs takes place outside the body as the cocoon moves forward over the body, picking up the eggs of one worm and the sperm of its mate.

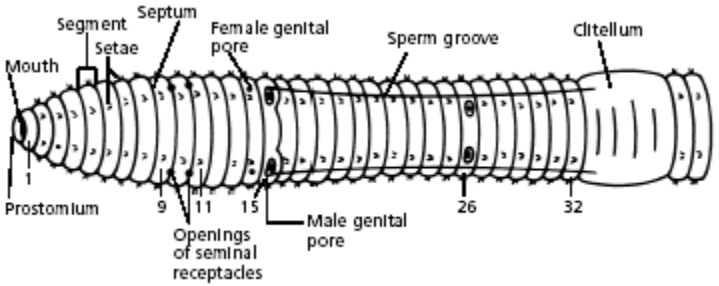
Circulatory system: The pumping organs of the circulatory system are five **aortic arches**. Circulatory fluids travel from the arches through the **ventral blood vessel** to capillary beds in the body. The fluids then collect in the **dorsal blood vessel** and reenter the aortic arches.

Nervous system: It consists of the **ventral nerve cord**, which

travels the length of the worm on the ventral side, and a series of **ganglia**, which are masses of tissue containing many nerve cells. The **nerve collar** surrounds the pharynx and consists of ganglia above and below the pharynx. Nervous impulses are responsible for movement and responses to stimuli. Each segment contains an enlargement, or **ganglion**, along the ventral nerve cord. Excretory functions are carried on by **nephridia**, which are found in pairs in each body segment. They appear as tiny white fibers on the dorsal body wall. The earthworm has no gills or lungs. Gases are exchanged between the circulatory system and the environment through the **moist skin**.

Procedure:

1. Place an earthworm in the dissecting tray & rinse off the excess preservative. Identify the **dorsal side**, which is the worm's rounded top, and the **ventral side**, which is its flattened bottom. Turn the worm ventral side up, as shown in the diagram below.



2. Use a hand lens as you observe all parts of the worm, externally and internally. Find the **anterior end** by locating the **prostomium**, which is a fleshy lobe that extends over the mouth. The other end of the worm's body is the **posterior end**, where the **anus** is located.

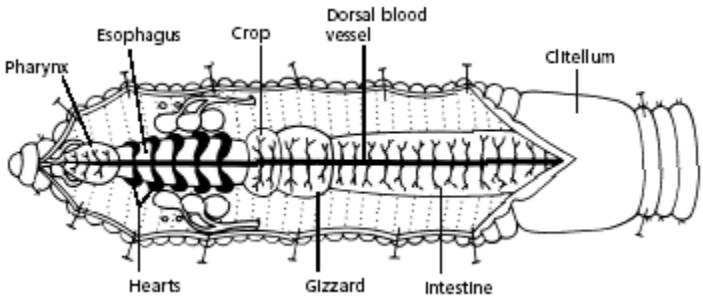
3. Locate the **clitellum**, which extends from segment 33 to

segment 37. Look for the worm's **setae**, which are the minute bristle-like spines located on every segment except the first and last one.

4. Refer again to the diagram of the ventral view of the worm to locate and identify the external parts of its reproductive system. Find the pair of **sperm grooves** that extend from the clitellum to about segment 15, where one pair of **male genital pores** is located. Look also for one pair of **female genital pores** on segment 14. There is another pair of male genital pores on about segment 26. Try to find the two pairs of openings of the seminal receptacles on segment 10. ***Note: These openings are not easy to see.***

5. Turn the worm dorsal side up. Using a scalpel and scissors, make a shallow incision in the dorsal side of the clitellum at segment 33. **CAUTION: *Scalpels and scissors are very sharp. Report any cuts to your teacher.*** Using the forceps and scalpel, spread the incision open, little by little. Separate each **septum** from the central tube using a dissecting needle, and pin down each loosened bit of skin. Continue the incision forward to segment 1.

6. Use the diagram below to locate and identify the five pairs of **aortic arches,** or hearts. Then find the **dorsal blood vessel**. Look for smaller blood vessels that branch from the dorsal blood vessel.



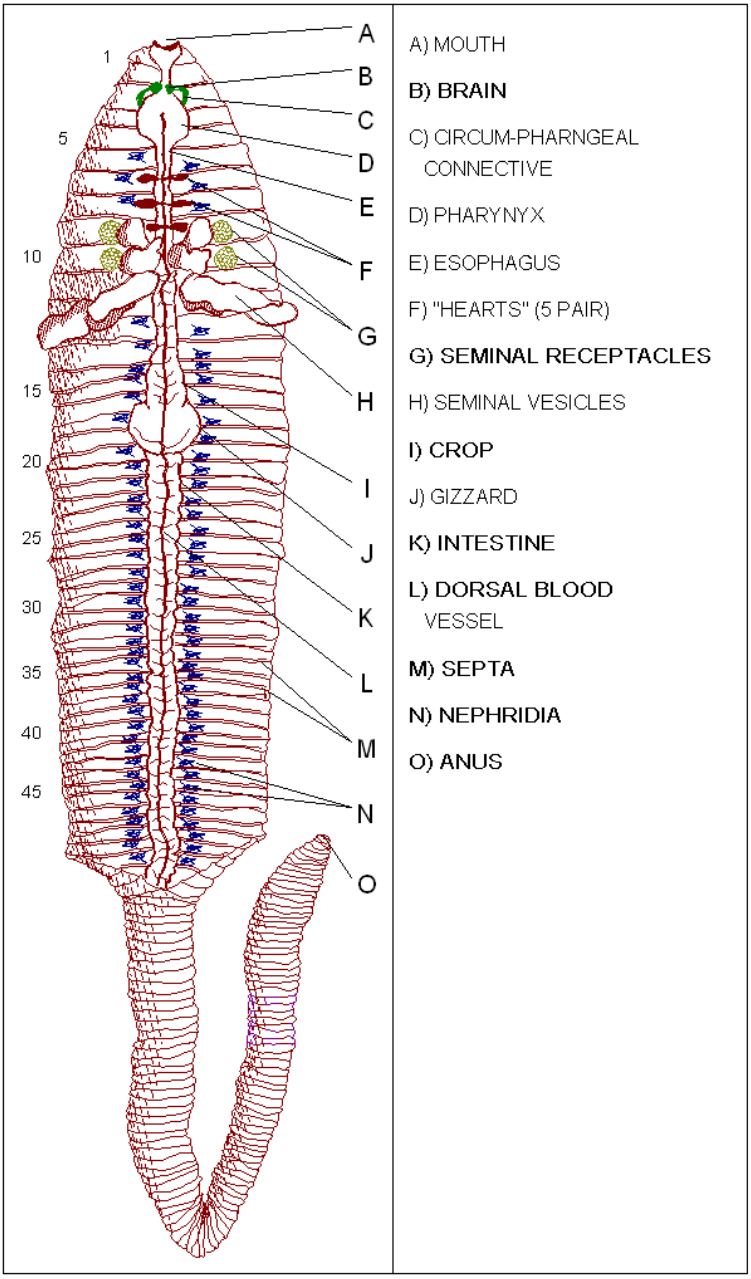
7. Locate the digestive tract, which lies below the dorsal blood vessel. Refer to the diagram above to locate the **pharynx**, **esophagus**, **crop**, **gizzard**, and **intestine**.

8. To find organs of the nervous system, push aside the digestive

and circulatory system organs. Use the diagram below to locate the **ventral nerve cord**. Trace the nerve cord forward to the nerve collar, which circles the pharynx. Find one pair of ganglia under the pharynx and another pair of ganglia above the **pharynx**. The **ganglia** above the pharynx serve as the brain of the earthworm.

9. The worm's excretory organs are tiny **nephridia**. There are two in every segment. Use the preceding diagram to locate some nephridia.

10. Clean up your work area and wash your hands before leaving the lab.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45
A) MOUTH
B) BRAIN
C) CIRCUM-PHARNGEAL CONNECTIVE
D) PHARYNYX
E) ESOPHAGUS
F) "HEARTS" (5 PAIR)
G) SEMINAL RECEPTACLES
H) SEMINAL VESICLES
I) CROP
J) GIZZARD
K) INTESTINE
L) DORSAL BLOOD VESSEL
M) SEPTA
N) NEPHRIDIA
O) ANUS

From: http://sps.k12.ar.us/massengale/earthworm_dissection.htm

Dissection of Yellow Perch (bony fish)

Introduction:

In this lab each student will work with a partner in order to learn from the dissection of a Perch. Dissection gives the student the opportunity to observe the placement of organs and their relationships to one another. Pictures and diagrams will aid in the completion of this lab dissection. Be thorough and do not rush through the lab. Read all directions carefully and make all **drawings as accurate and neat** as possible.

Materials:

Preserved perch
Dissecting tray
Dissecting probe and scissors

Procedure

A. External Anatomy of Perch:

1. Place a preserved perch on a dissecting tray. Locate the head region. Examine the eyes.
2. Are there any eyelids present? _____.
3. Examine the 5 types of fins. Each fin has a purpose or job, what is the purpose of the following fins?

a). caudal fin_____
b). dorsal fins_____
c). pectoral fin _____
d). anal _____

e). pelvic _____

4. Locate the lateral line. Using the hand lens and look at the line and the surrounding area.

B. Internal Anatomy of Perch:

5. Using your thumb, lift up the edge of the operculum and raise it up as far as you can. Using your scissors, cut the operculum off as close to the eye as possible. You have exposed the gills. The gill are layered one on top of another. Using your probe, carefully lift each of these layers.

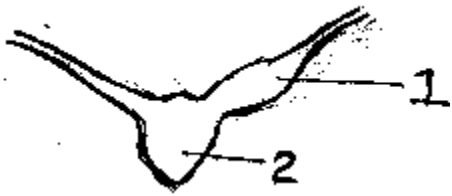
6. How many layers do you find? _____

7. Using your scissors, remove one of these layers. Examine the feathery structure.

8. To expose the internal organs you will cut away part of its muscular wall. Grasp your fish, holding it with your thumb on one side and fingers on the other. Turn your hand upward to expose the ventral surface. Using your scissors, insert the point into the skin just in front of the anus. Cut forward to the gills. Be careful not to destroy any of the internal organs since they are mostly found in this area.

9. The fish contains a 2 chambered heart. Locate this organ found just behind and below the gills.

Label the parts of the heart below.



Fish Heart

10. Part 1 _____ Function: _____

11. Part 2 _____ Function: _____

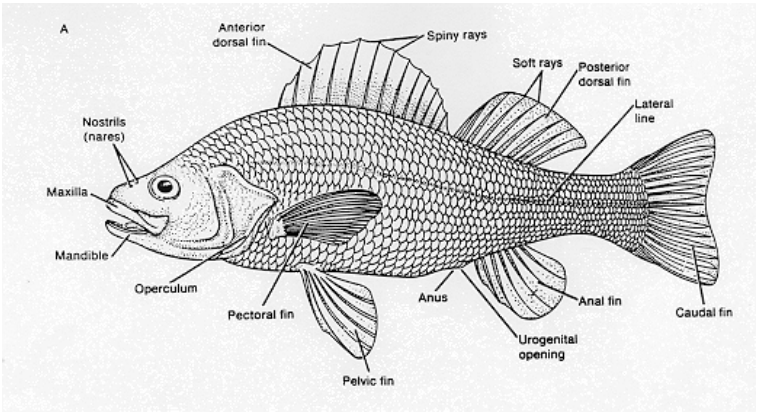
12. Locate the tube-like digestive system. Begin just behind the mouth in the area called the pharynx. This area leads into the gullet or the opening of the esophagus. The esophagus leads into the stomach. Cut out the stomach and split it open.

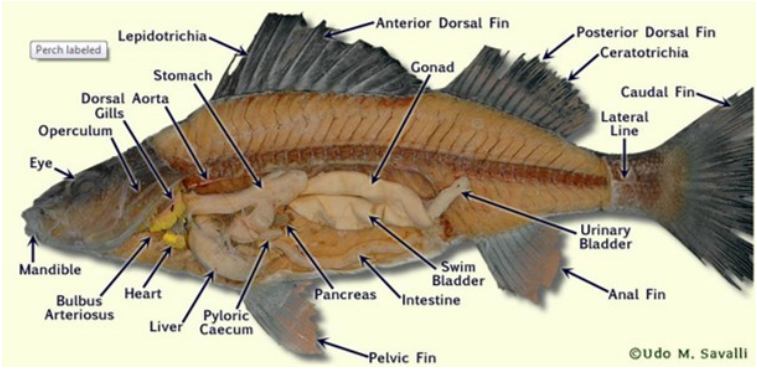
13. Locate the rather large liver located just in front of the stomach.

14. Try to locate the kidneys, found just below the spinal column. Their main function is to rid the body of nitrogenous waste.

15. The swim bladder is the last remaining organ to be identified. It is located between the kidneys and gonads.

16. What is the function of the swim bladder? _____





Dissection of Rat (mammal)

Introduction:

Each student will work with a partner to learn about the anatomy and physiology of a rat. Dissection of the rat offers the opportunity to compare the anatomical differences between the annelid and bony fish to the mammal. The rat serves as a model of anatomical and physiological similarities to humans. Be thorough in following directions of this dissection.

Materials:

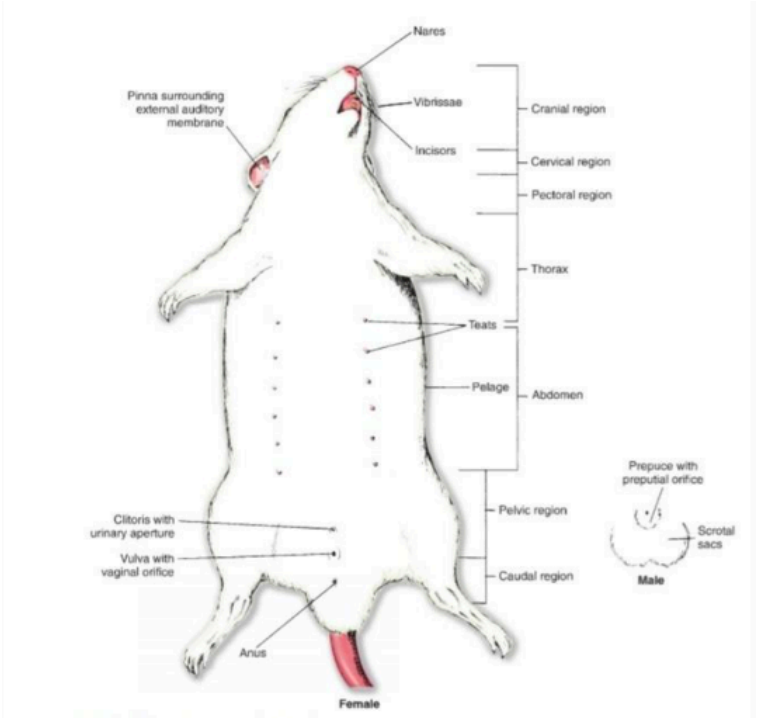
Preserved rat
Dissecting tray
Dissecting probe and scissors

Procedure

A. External Anatomy of Rat:

1. Place the preserved rat specimen in a dissecting tray, ventral side up. Locate the following regions of the body: cranial, cervical,

pectoral, thorax, abdomen, pelvic and caudal regions. Identify external features.

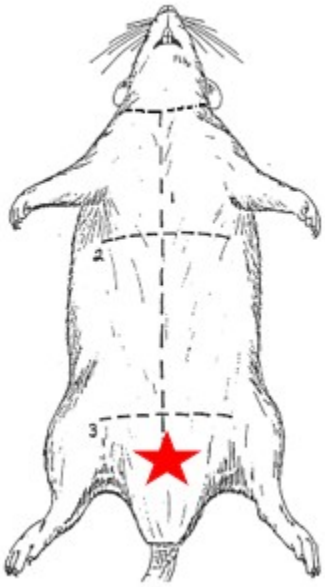


2. Determine the sex of the rat specimen by examining the pelvic region. State whether the specimen is male or female.

B. Internal Anatomy of Rat

4. Tie one end of a piece of string around the right front paw, wrap the string around the outside of the dissecting, and tie the other of the string to the left front paw. Do the same for the hind paws.

5. Using the below image as a guide, make cuts starting at the star and move upward toward the cranial region. Open up the pectoral, thorax, and abdominal regions by pinning the flaps to the dissecting tray.



6. Using the below image, examine and identify the digestive organs. Notice the scattered cream colored fat deposits. Note the size of the liver in proportion to the abdomen.

7. Remove the digestive system by cutting the esophagus above its connection with the stomach (below the diaphragm) and by cutting the rectum. Notice the way the small intestine and large intestine are twisted over one another.

8. Remove the liver carefully and compare the color of the spleen and liver. Note there is no gall bladder in the rat.

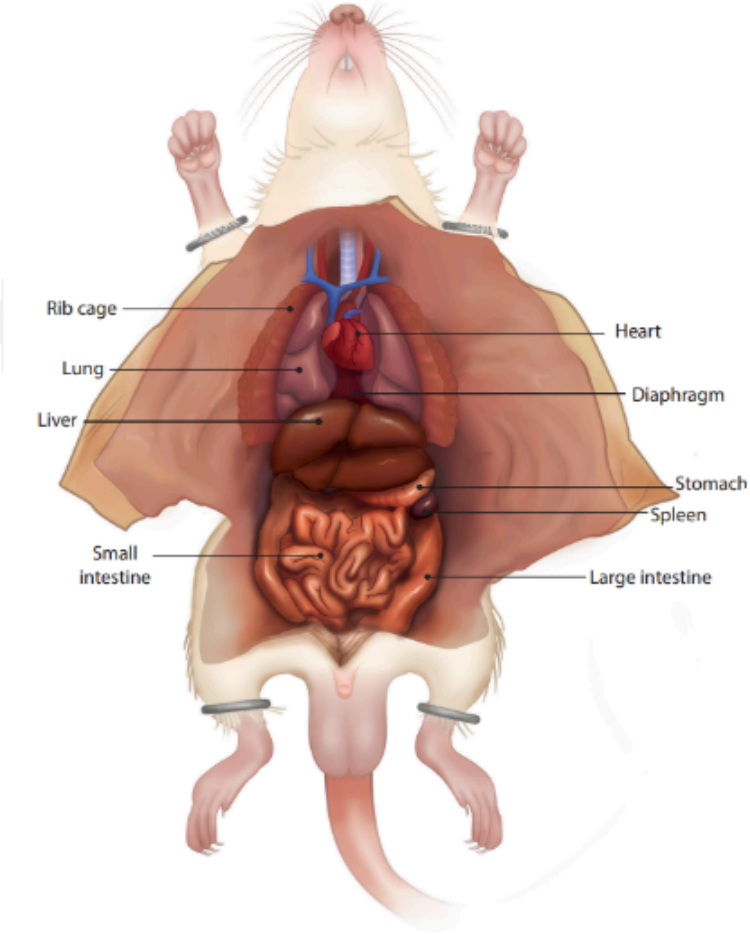
9. Observe the exposed kidneys and associated urinary organs, and the reproductive system left behind in the abdominal cavity.

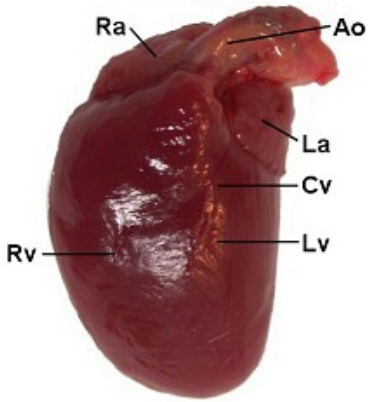
10. Examine the thoracic (pectoral) region by cutting away the ribs to reveal the heart surrounded by a thin membrane called the pericardium, the whitish thymus gland that lies directly over the

upper part of the heart and the lungs that lay either side of the heart.

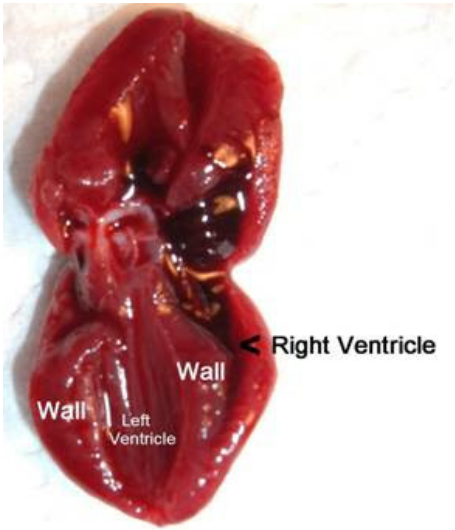
11. Remove the heart and thymus to locate the trachea, which is identifiable by its rings of cartilage. The esophagus lies just underneath the trachea.

12. Make a coronal incision into the heart (divides the heart into anterior and posterior regions) to view the 2 ventricles. Note the heart of the rat has 4 chambers: 2 atria and 2 ventricles.





Heart (Ao: aorta, Cv: conoventricular vein, La: left auricle, Lv: left ventricle, Ra: right auricle, Rv: right ventricle).



Dispose of your rat and its removed parts by placing in the bagged

bucket. Clean up your work area by washing dissecting tools and tray.

Lab 6: Frog Leg Muscle Contraction

In this lab activity, you will learn how to isolate the gastrocnemius muscle of the frog and its somatic motor nerve (sciatic nerve) and record the contractile responses resulting from an applied electrical stimulus to understand how neuronal impulses can elicit muscle contractions.

Learning objectives for this lab activity:

1. Prepare a pithed frog for the study of muscle physiology.
2. Describe how muscle can contract when an electrical stimulus is applied.
3. Explain how motor nerves stimulate the contraction of skeletal muscles.
4. To record the twitch Threshold, maximal twitch response, summation, tetanus and fatigue for frog skeletal muscle and its motor nerve.
5. Examine the effects of the length of the sarcomere on the force of contraction.

Background:

The ability to move is a basic property of life permitting an animal to respond to its environment. Depending upon the structural complexity of the organism, the mechanisms for motion can appear very different. For example, amoeboid movement, ciliary and flagellar motion, and muscular activity all appear very diverse. However, upon close inspection, the basic molecular mechanisms are very similar involving almost identical chemicals but in different spatial arrangements. **For metazoan, contractile structures are organized into individual or groups of cells known as muscle fibers. Myocytes can efficiently transform chemical energy into a mechanical force that is capable of work.** Muscle, perhaps more than any other physiological system, has been studied thoroughly.

Of the three muscle types, smooth, cardiac and skeletal, the later is best understood. We know more about skeletal muscles at all levels of organization than any other system. In this laboratory, we will look at the contractile properties of the **gastrocnemius muscle** from the lower leg of the grass frog, *Rana pipiens*. Muscle tissues are relatively easy to work with experimentally. They can be removed from an organism intact with little loss of function. Also, muscle tissue can be taken from an organism without removing other noncontractile tissue. **Since muscular contractions are large in magnitude, they can be accurately measured with simple recording equipment.**

For *in vitro* contraction studies, there are two major preparations: 1) **isotonic** and 2) **isometric**. In the former, isotonic, the muscle can move exerting a constant force as the fiber changes length. In the latter, isometric, the length of the muscle is held constant as the force of contract varies. The physiology of contraction can be studied in muscles isolated from a pithed frog. The preparation can be stimulated directly by an electric shock or indirectly through activation of the appropriate motor nerve. **In the gastrocnemius muscle experiments described here, we will examine properties of isotonic contractions.**

Stimulation of Motor Nerve

In the vertebrates, skeletal muscle contractions are evoked in vivo by impulses in somatic motor neurons. The soma or cell body of motor or efferent neurons is located in the ventral gray horn of the spinal cord (Figure 1).

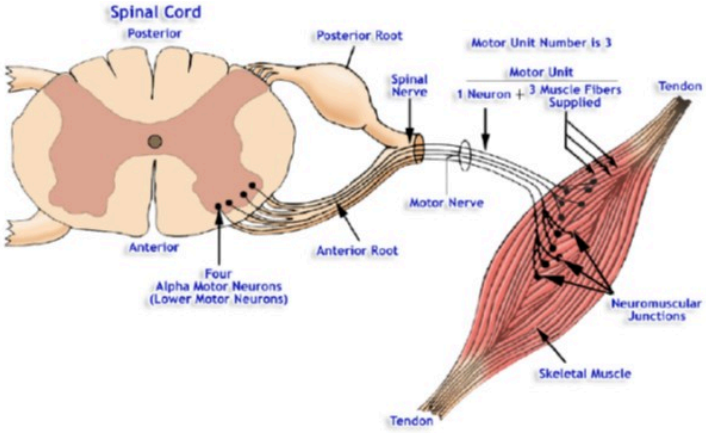


Figure 1

Normally, electrical activity in the somatomotor fibers originates in the spinal cord and travels over a single axon to a myocyte or to a group of myocytes known as a motor unit. The **motor neurons** are activated either by other neurons in the brain or spinal cord, or by local **sensory neurons**. Impulses in efferent neurons may also be stimulated by damaging fibers peripherally. This damage produces an injury current, which stimulates action potentials. This is why a **muscle twitch** occurs when a nerve is pinched. Once the axon of motor neurons leaves the spinal cord, it joins a **nerve** along with hundreds of other motor and sensory neurons. Each efferent somatic neuron transmits impulses from the spinal cord directly to a specific group of myocytes evoking contraction. The form of the contraction expressed by skeletal myocytes, or motor unit depends upon the pattern of motor neuron stimulation. For example, the gastrocnemius is innervated by hundreds of motor neurons in the **sciatic nerve**. Each motor neuron has a **threshold voltage** for activation. If a neuron is stimulated with a single, small **supra-threshold voltage**, a single **all-or-none contraction** occurs in all myocytes of a motor unit. This is a **twitch**. When more neurons are activated by increasing stimulus strength, additional motor

units contract increasing muscular strength, and the twitch is larger. When all efferent neurons are activated for a muscle, a maximum-sized twitch occurs indicating all motor units have been **recruited**. **Supra-maximal voltages** can recruit no additional motor units and do not increase muscular strength. This relationship between neuron stimulation, motor unit activation, and muscle contraction is known as **excitation-contraction coupling**. Because increasing stimulus amplitude recruits more motor units, this pattern or kind of response is known as **spatial** or **motor-unit summation**.

In addition to recruitment of motor units by increasing stimulus amplitude (i.e. voltage,) variation in contraction can be achieved by **temporal summation**. Action potentials in the motor nerve fibers elicit the release of a chemical neurotransmitter called **acetylcholine** (ACh) from the axon endings. This transmitter combines with **nicotinic receptors** to produce action potentials on the membrane of the skeletal myocyte. An electrical impulse in the muscle cell, in turn, causes Ca++ to be released from the **sarcoplasmic reticulum** (SR). At the molecular level, for contraction, the release of Ca++ into the cytosol permits actin and myosin to interact in the presence of ATP. Actin-myosin interactions and contractions are relaxed when Ca++ is re-absorbed into the SR by a Ca++ pump.

The **rate** at which myocytes are stimulated influences how long Ca++ lingers in the cytoplasm. If a myocyte receives a single, sub-maximum stimulus, Ca++ is released and quickly re-absorbed. If the myocyte receives a second impulse before relaxation is fully affected, the amount of cytoplasmic Ca++ in the second contraction is greater than the first. Consequently, the strength (i.e. force in grams) of the 2nd contraction is greater (Figure 2).

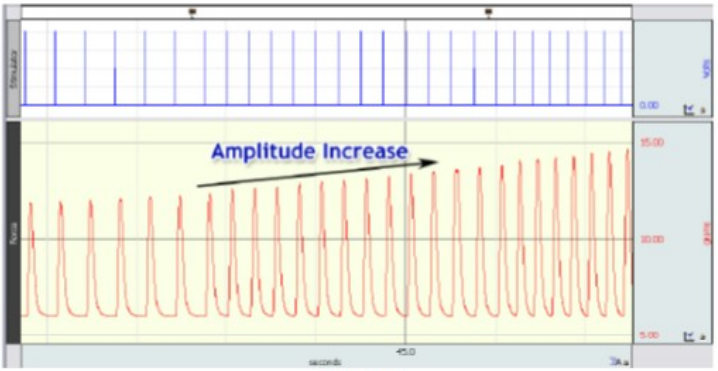


Figure 2

If no other impulse appears, all Ca++ is returned to the SR. If a train or volley of sub-maximal impulses is used to stimulate a myocyte, the strength of the contraction is proportional to the frequency of stimulation. At the maximum rate, the myocyte remains in a persistent, sustained contraction known as **tetanus**. If the rate of stimulation (pulses/second) is increased above the **tetanizing frequency**, no greater increase in strength occurs. At frequencies of stimulation between initial temporal response and tetany, contractions express partial relaxations. From its appearance, this response is called **treppe** or **stair-step**. In temporal summation, by varying the stimulating frequency we manipulate cytoplasmic Ca++ and molecular interactions within motor units to control the strength of contraction.

For the frog gastrocnemis, there are two modes or ways to simulate the muscle with electrodes. The way most resembling a physiological state is to apply an exogenous electrical signal indirectly via the sciatic nerve. Alternatively, the electrodes can be inserted directly into the muscle which applies the stimulus directly to the myocytes. **If you are able to activate muscular contraction via the sciatic nerve, DO NOT use excessively large supra-maximal stimuli. These large voltages will destroy the neurons.**

In striated myocytes, contractile proteins are assembled in linear, parallel arrays stabilized by **z-discs**, **titan** and **nebulin** in each **sarcomere**. Sarcomeres are arranged in a series form myofibrils, hundreds of which fill the **sarcoplasm** of the muscle cell. When Ca++ is abundant in the sarcoplasm, actin and myosin bind forming the **rigor complex**. In the presence of ATP, the two molecules cycle continuously between the bound and unbound states. As a consequence, filamentous actin and myosin macromolecules slide past each other in opposite directions producing the force of contraction. When Ca++ is removed, *in lieu* of the ATP concentration, the **actinomyosin** complex is blocked and the rigor complex relaxes. The force generated during contraction of a sarcomere is related to its shape. If a myocyte is extirpated from tendons and elastic components of the fascia or **aponeuroses** contract, thick and thin filaments overlap maximally in each sarcomere. When the myocyte is stimulated, no force is generated. On the other hand, if the fiber is stretched to its limits just before tearing, actin and myosin filaments do not overlap. Again no force can be generated by the fiber with stimulation. At intermediate lengths, the thick and thin filaments overlap to various degrees depending upon degree of stretch. Thus, muscle strength (in grams) is related to length (in mm) in a very precise way. A plot of gastrocnemius muscle **force versus length** will illustrate an optimum length of the muscle for generating a force. This is usually a dome-shaped curve with maximum force generated at an intermediate length. *In situ*, skeletal muscles are usually stretched to intermediate length by ligaments anchoring them to bone so they can generate maximum force if it is desired.

Fatigue is a physiology state commonly observed in biological preparations. It is a decline or decrease in the ability to respond when repeatedly presented the same stimulus. In muscle preparations, fatigue can occur for several reasons. First, impulse frequency in motor neurons can decrease. Recall that the action potentials in neurons and myocytes are all-or-none signals, reoccurring with the same amplitude (or height). Since the

frequency of impulses regulates amount of neurotransmitter released, a decline in neuronal impulse frequency results in fewer impulses and less neurotransmitter to the myocyte. Second, the neuron and myocyte communicate via a synapse at the **neuromuscular junction** (or endplate).

If the pre-synaptic membrane does not replace vesicle containing neurotransmitter, the ability to produce a post-synaptic impulse in the myocyte will decrease. And third, the myocyte can become less responsive with prolonged stimulation. This occurs by either of two ways, decrease in Ca++ availability or decrease in ATP (or other energetic molecules). Since it is abundant is external solutions and stored in the sarcoplasmic reticulum, Ca++ depletion occurs infrequently and is not usually related to fatigue. However, a sudden influx Ca++ without an impulse produces a spontaneous twitch known as a "**spasm**" or "**cramp.**" Most often fatigue results from a decline in the availability of biological energy. When a myocyte is stimulated rapidly to tetany, ATP is consumed rapidly sustaining the process. Since the myocyte manufactures ATP at a finite rate, energy supply cannot match demand and consumption. Consequently, when stimulation produces tetany, fatigue results from a decline in available ATP. If tetany is interrupted for a short period (few seconds), energy production will restore maximum muscular performance. You can observe this in your gastrocnemis preparation.

In the following experiment, the frog sciatic and gastrocnemius will be exposed to study basic physiological properties of muscle contraction. Initially, you will determine Threshold and examine the components of muscular contraction: **latent, contraction** and **relaxation phases** or periods. Also, you will study **motor unit summation** and **temporal summation** by independently changing stimulus amplitude and frequency while observing the effect on muscle responses. Within the vertebrate body, fine motor control is a product of the simultaneous **integration** of temporal and spatial summation (Figure 3).

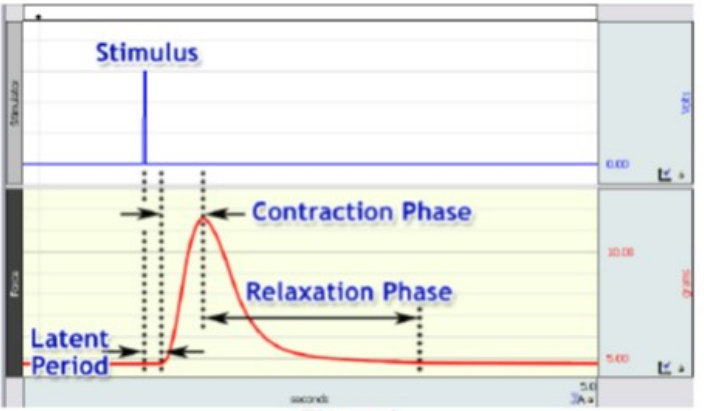
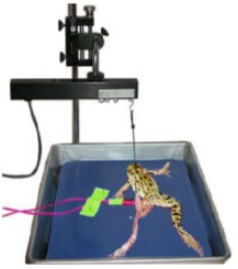
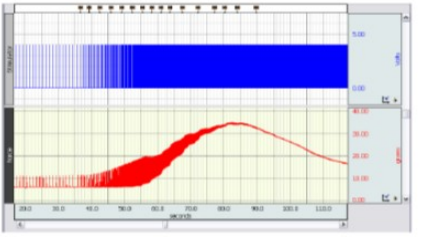


Figure 3

Experimental setup:





Experiment AM-10: Summation, Tetanus, and Fatigue in an Intact Nerve-Muscle Prep

By: RJ Cooper, Ph.D., Margaret A. Weck, D.A., and Dayton J. Ford, Ph.D.: at the St. Louis College of Pharmacy. Adapted from: Hoff, H.E. and L.A. Geddes, Experimental Physiology (1965)

Equipment Required

PC or Mac Computer

IWX/214, USB cable, IWX/214 power supply
FT-104 Force transducer
C-STIM-BNC-N2 Needle-type stimulating electrodes
C-BNC-SE Sleeve-type stimulating electrodes
Female BNC to Dual Banana Adapter
Ringstand and clamps
Muscle tension adjuster
Thread
Frog board
Dissection tray
Glass dissection hooks
1cc tuberculin syringe with needle
Amphibian Ringer's solution (see Appendix)
1% Tubocurarine solution in Ringer's

For details of the setup please see the "FrogNerveMuscle_Setup214" PDF document.

Procedure:

Start the Software

1. Click on LabScribe
2. Click Settings _ Animal Muscle _ FrogNerveMuscle

Exercise 1: Twitch Threshold Determined by Direct Stimulation

Aim: To determine the threshold stimulus of the muscle when it is stimulated directly with single pulses of constant duration.

Approximate Time: 15 minutes

• Plug in the Pin stimulator into the SI port, Leave the sleeve stimulator on the nerve but not plugged in to the IWORX box
• DO NOT PIN THE FOOT, Pin skin around the knee

1. Click the Stimulator Preferences icon on the LabScribe toolbar (Figure AM-10-L1) to open the stimulator control panel (Figure AM-10-L2) on the Main window.

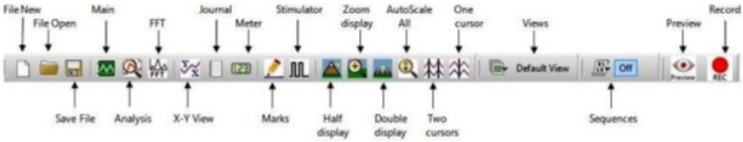


Figure AM-10-L1: *The LabScribe toolbar.*

2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window:

• the pulse amplitude (Amp) should be set to 0.000 V;
• the number of pulses (#pulses) to 1;
• the frequency (F(Hz)) to 1;
• and, the pulse width (W) to 5 ms.
• Click APPLY in the upper left of the control panel

3. The value for a stimulus parameter can be changed by either of two methods: click on the arrow buttons to the right of the window that displays the value of the parameter to increase or decrease the value; or, type the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize the change in any stimulus parameter.

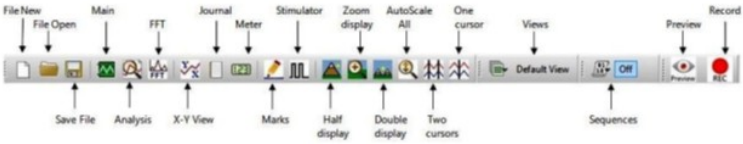


Figure AM-10-L2: *The stimulator control panel*

4. Type 0.000V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.000V and press the mark button to attach the comment to the recording.

5. Apply a light load to the muscle by raising the force transducer with the tension adjuster until the trace moves a few mV above the baseline. If you are using a FT-302 force transducer, the initial baseline can be adjusted to 0 mV by rotating the offset knob on top of the FT-302 transducer.

6. Once a light load is applied to the muscle, Click on the Stop.

7. Change the stimulus amplitude (Amp) to 0.1V using one of the techniques described in Step 2. Click the Apply button on the Stimulator control panel to effect the change in amplitude.

8. Type 0.1V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.1V and press the mark button to attach the comment to the recording. Stop the recording as soon as the muscle twitch is finished, or after a couple of seconds if no twitch is detected.

Note: Be sure to have at least one member of your lab group LOOKING at the specimen at all times. It is possible for contractions to occur and not be recorded. Click AutoScale to make sure the recording is displayed properly. Check the tautness of the thread going from the tendon to the transducer, the transducer itself, and check the connection from the transducer to the iWorx recorder.

9. Determine the threshold voltage for direct muscle stimulation by increasing the stimulus voltage in 0.1V increments. DON'T SKIP A VALUE and DO NOT EXCEED 1V. Remember to click the Apply button on the Stimulator control panel each time it is changed. Record and mark the muscle response as described in Steps 3 and 7.

10. Once the threshold voltage has been determined, test the muscle's response to direct stimulation at additional stimulus voltages (Figure AM-10-L3). Increase the stimulus amplitude in 1.0V increments, up to 5 V. Record the muscle response and mark the recording as described in Steps 3 and 6.

11. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

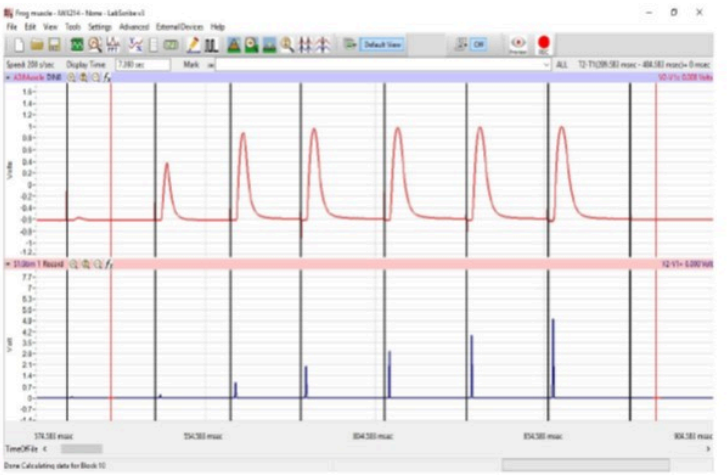


Figure AM-10-L3: *Muscle twitches resulting from direct stimulation of the muscles.*

Exercise 2: Twitch Threshold Determined by Nerve Stimulation

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated by single pulses of constant duration.

Approximate Time: 30 minutes

1. On the iWorx box, disconnect the BNC connector of the pin electrodes used for direct

stimulation of the muscle. Leave the pin electrodes in place in the muscle.

2. On the iWorx box, connect the BNC connector of the sleeve electrodes to the BNC-banana adapter on the stimulator output of

the IWX/214 or to the BNC output of the stimulator on the front of the IXTA.

3. Reset the pulse amplitude (Amp) to 0.000V. Remember to click the Apply button on the stimulator control panel to finalize the change.

4. Repeat Exercise 1 while stimulating the gastrocnemius muscle through the sciatic nerve with single stimulus pulses of increasing amplitude (Figure AM-10-L4). Label the recording at each voltage attempted.

5. Determine the threshold voltage for nerve stimulation by increasing the stimulus voltage in 0.1V increments. Above threshold, test the muscle's response to nerve stimulation in 1.0V increments.

6. Click the Save button to save the file.

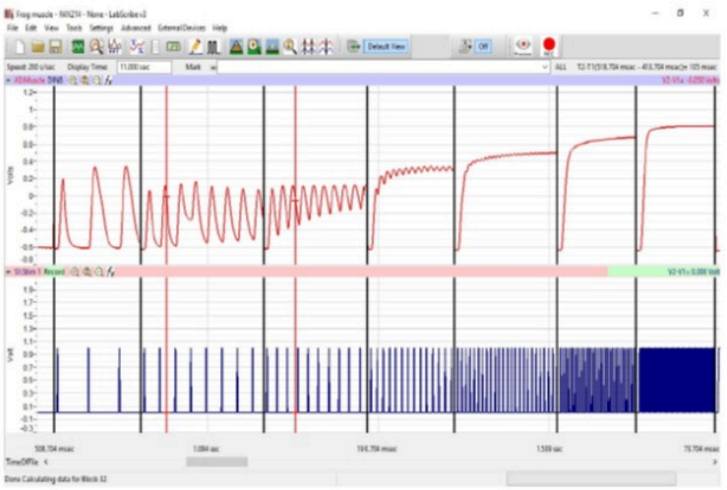


Figure AM-10-L4: Muscle twitches resulting from nerve stimulation.

Exercise 3: Effect of Stimulus Frequency on

Twitch Amplitude

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated for short periods at different stimulus frequencies.

Approximate Time: 30 minutes

1. On the iWorx box, leave the BNC connector of the sleeve electrodes attached to the BNC banana adapter on the stimulator output of the IWX/214 or on the stimulator output on the front of the IXTA.

2. Set the stimulus frequency (F(Hz)) to 2 Hz, the number of pulses (#pulses) to zero (0), and the pulse amplitude (Amp) to the lowest value that still gave strong contractions in the previous exercise. This voltage will usually be in the range of 0.400V to 1.000 V, but your frog may be different. Keep the pulse width (W) at 5 ms. Remember to click the Apply button on the Stimulator control panel with each change.

***Warning*: Since the number of pulses (#pulses) is set to zero (0), the stimulus pulses will be delivered continuously at the frequency selected. It is important to stop recording once you see the effect of stimulating at a certain frequency.**

3. Type 2 Hz in the Mark box to the right of the Mark button. Click Record to stimulate the nerve and click the mark button to mark the recording. Stop the recording after 5 seconds

4. Change the stimulus frequency to 4 Hz. Repeat Step 3. Continue the recording for stimulus frequencies of 5, 10, 25, 50, and 100 Hz (Figure AM-10-L5).

5. Click the Save button to save the file.

6. When you analyze the data for this exercise, note the frequency at which wave summation begins, the frequencies at which incomplete and complete tetanus occur, and the shape of the contraction waves and any indication of fatigue.

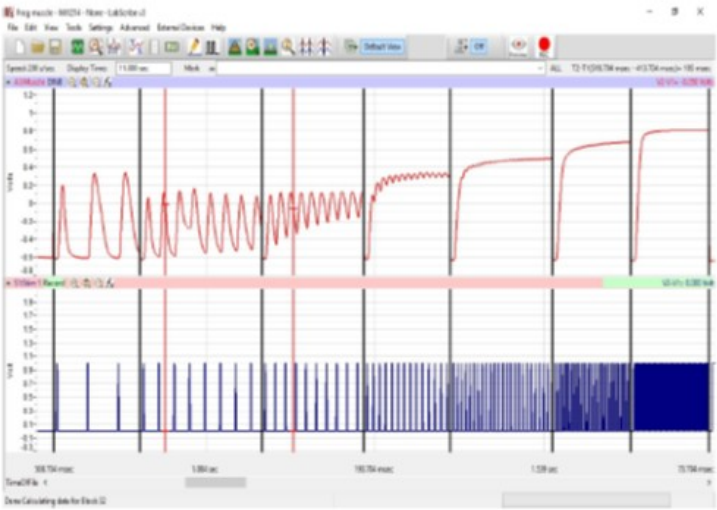


Figure AM-10-L5: Muscle response from nerve stimulation with increased stimulus frequencies.

Exercise 4: Fatigue with Repeated Stimuli

Approximate Time: 45 minutes

1. On the iWorx box, disconnect the sleeve electrodes from the stimulator output, and connect the pin electrodes to the stimulator. Leave the sleeve electrodes in place on the nerve.

2. Set the Display Time to 20 sec, the stimulus frequency to 4 Hz, the number of pulses (#pulses) to zero (0), and pulse amplitude (Amp) to an adequate value between 0.4V and 1 V. Remember to click the Apply button.

3. Type 4Hz-Direct in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 4 Hz and press

the mark button to attach the comment to the recording. Stop the recording after 5 seconds

4. After recording at 4Hz with the pin electrodes, switch to the sleeve electrodes.

5. Type 4Hz-Nerve in the Mark box to the right of the Mark button. Click on the Record button to stimulate the nerve with 4 Hz and press the mark button to attach the comment to the recording. Stop the recording after 5 seconds.

6. Change the stimulus frequency to 50 Hz. Remember to click the Apply button on the Stimulator control panel.

7. Type 50Hz-Nerve in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 50Hz and press the mark button to attach the comment to the recording. Continue to stimulate through the nerve until the force of the muscle contraction drops to an amplitude that is only 25% of the maximum force at the beginning of this tetanic contraction.

8. Continue to record after the contraction force of the muscle has dropped below 25% of maximum. Quickly disconnect the BNC connector of the sleeve electrodes from stimulator, and reconnect the BNC connector of the pin electrodes to the stimulator.

9. Stimulate the muscle directly for 3 seconds, with the same voltage, duration, and frequency that was used to fatigue the muscle.

Note: If no contraction is seen while using these stimulus parameters, increase the stimulus amplitude until a contraction is measured.

10. Continue to record after the direct stimulation of the muscle. Quickly disconnect the BNC connector of the pin electrodes from the stimulator, and reconnect the BNC connector of the sleeve electrodes to the stimulator.

11. Apply the same stimulus used in Step 9 to the nerve for a period of 3 seconds.

12. After you stop the recording, label the recording with marks and notations to indicate the site of stimulation (muscle or nerve) and the stimulus voltage that generated each response.

13. Click the Save button to save the file.

14. When you analyze the data for this exercise:

• Note the shape of the contraction waves and any indication of fatigue.

• Note whether the muscle contraction was stronger when the stimulus was applied directly to the muscle or when the stimulus was applied to the nerve. What does this indicate about the location of the fatigue observed during nerve stimulation?

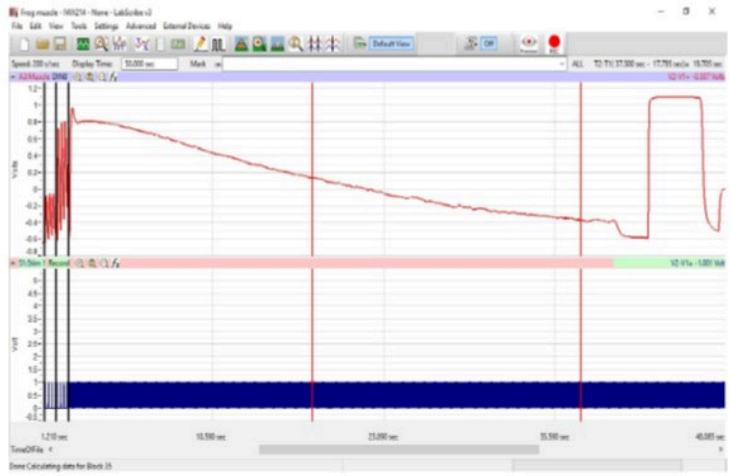


Figure AM-10-L6: Decrease in muscle force (fatigue) with continuous, high frequency stimulation through the nerve innervating the muscle. The period of fatigue occurring during nerve stimulation is followed by a large response by the muscle when it is stimulated directly.

Note: As you complete each exercise, make sure to record your data and answer the questions in the lab report document.

Lab 7: Oral Presentations on Comparative Physiology

Oral presentations on Comparative Physiology
Oral presentation grading rubric (25 points)
Name of student:_____Date:_____

Content of presentation – contains background information about the organisms chosen, physiological system with an explanation of how this system functions and has adapted to then links this to its survival. Compares the physiological system across three organisms, importance of the analysis for biological research and how it can potentially benefit huma
Delivery of presentation – a good amount of eye contact, body language is appropriate, lou refers to slides or poster when discussing relevant points, good transitions between slide presentation flows well. The presentation is no more than 15 minutes.
Visual appeal of presentation – the slides/poster are well balanced and visually appealing, contrast and the text is easy to read, the figures are formatted so they are easy to read. Th identify the separate parts of the presentation.
Elevator talk – the talk is gauged for a general audience, it is succinct, clear, well-rehearse overview an importance of the comparative analysis. The talk lasts around no more than 2
Literature cited – references properly formatted in a consistent scientific journal style, thi end of the talk as full references.
Answering questions – is able to give some sort of educated answer to the questions asked additional reading of previous literature, questions are asked when others are presenting.

Lab 8: Crayfish Heart & Blood Physiology

Materials needed for blood physiology activity:

Fan for blood drying
Glass slides
Giemsa staining solution (mix 1 part Giemsa stock solution with 9 parts phosphate buffer)
Compound light microscopes
Methanol
pH 7.2 phosphate buffer
Immersion oil
5 µl microcapillary with bulb for collecting crayfish hemolymph (one per crayfish)
Coplin staining jar (2 units)

Background

Cardiac muscle contraction is an involuntary process driven by electrical signals from the sinoatrial (SA) node that causes coordinated heart muscle cell (cardiomyocyte) contractions. This process, called excitation-contraction coupling, begins when an electrical action potential opens L-type calcium channels, allowing calcium to enter the cell. This initial calcium influx then triggers a larger release of calcium from the sarcoplasmic reticulum through the calcium-induced calcium release (CICR) mechanism. The increased intracellular calcium binds to troponin, causing the protein to move and expose binding sites on actin filaments, allowing myosin heads to bind and slide the filaments to shorten the sarcomere and create muscle contraction.

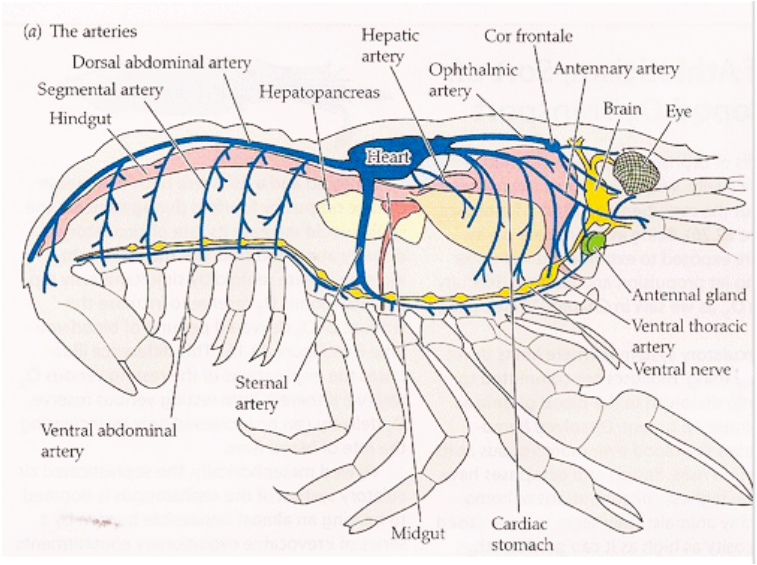
The process of cardiac muscle contraction:

- **Electrical impulse:** An electrical action potential is generated by the SA node and spreads through the heart via gap junctions

in specialized conductive fibers.

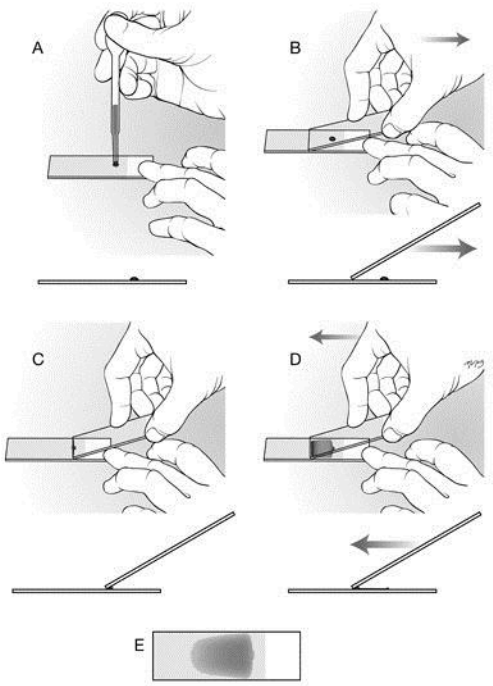
- **Calcium influx:** The action potential travels along the cardiomyocyte's cell membrane (sarcolemma) and down the T-tubules, triggering L-type calcium channels to open and allowing calcium from the extracellular fluid to enter the cell.
- **Calcium-induced calcium release (CICR)**: The small amount of incoming calcium activates ryanodine receptors on the sarcoplasmic reticulum, causing a much larger release of stored calcium into the cytoplasm.
- **Crossbridge cycling:** The surge of calcium binds to troponin C, causing it to move tropomyosin and expose binding sites on actin filaments. Myosin heads then bind to these sites, and using ATP, they pull the actin filaments toward the center of the sarcomere, resulting in muscle contraction.
- **Relaxation:** The action potential ends, calcium influx stops, and the cell actively pumps calcium back into the sarcoplasmic reticulum and out of the cell. This reduces the intracellular calcium concentration, causing calcium to detach from troponin, which allows tropomyosin to cover the actin binding sites again and the muscle to relax.

In this lab, you will measure the crayfish heartbeat and understand how it can be modulated by drugs acting on the nervous system. In addition, you will conduct a blood smear to inspect the cell contents of the hemolymph and compare these with the cell contents of blood.



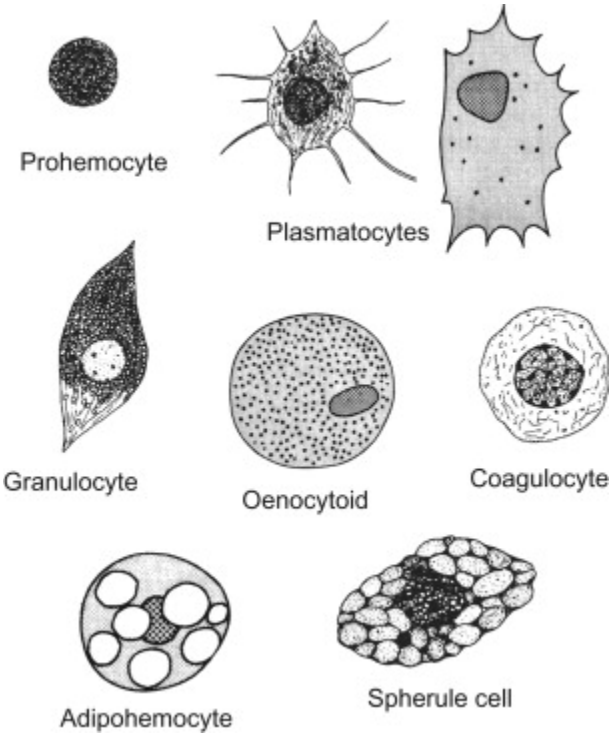
Crayfish blood analysis

1. Collect a blood sample from the caudal blood vessels or by cutting the tail when you are preparing the crayfish for the heart activity.
2. Place a drop of blood on a clean microscope slide.
3. Spread the drop into a thin smear using another slide held at a 45-degree angle.

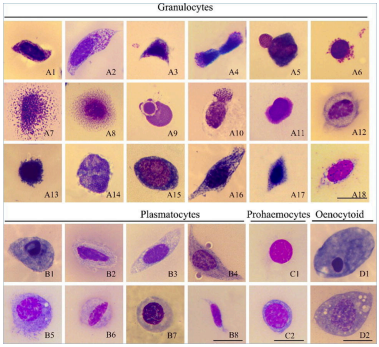


4. Blood smearing technique using two glass slides.
5. Set this aside to allow the smear to air dry for 30 min.
6. Fix the slides by immersing them in methanol for 1 minute. Let it air dry for 30 seconds.
7. Place the slides in the working Giemsa solution for 20-30 minutes. Place the slide in a staining jar and flood the smear with the prepared Giemsa stain solution.
8. Gently rinse the slide by dipping it in pH 7.2 phosphate buffer, followed by a rinse with distilled or neutral water. Be careful not to wash away the smear.
9. Stand the slide upright and let it air dry completely for 10 min. Once dry, add a drop of immersion oil and examine the

stained hemolymph cells under a microscope with the 100x oil immersion lens.



Prohemocytes	– Small, rounded cells – Large nucleus and basophilic cytoplasm – Conside that can differentiate into other hemocyte types
Plasmatocytes	– Most common type of hemocyte – Spindle or leaf-like shape with long, h or filopodia) – Actively involved in phagocytosis, encapsulation, and nodula
Granular Hemocytes (Granulocytes)	– Round or oval with numerous cytoplasmic granules – Possess macropha phagocytosis and forming "sticky nets" to trap pathogens – Involved in cell encapsulation and nodulation
Coagulocytes	– Contain cytoplasmic granules – Their main function is to participate in t wounds
Oenocytoids	– Large, often irregular or round in shape – Typically have a round, "fried e nucleus and cytoplasmic granules – Not involved in phagocytosis – Functio are involved in some immune responses



Use the pictures and descriptions above as guides. Draw and label what you see underneath the microscope in your lab report, while also including the total magnification you are viewing the blood sample at.

The Crayfish Heart Activity

Background

Vertebrate hearts are myogenic; each muscle cell has intrinsic pacemaker properties. Each cell will beat in the absence of any neural input. The cells throughout the heart beat in a coordinated fashion because they are electrically coupled and beat at the same rate as the pacemaker cells in the sino-atrial and atrial-ventricular nodes. In most vertebrates, the blood is pumped into a closed circulatory system of arteries and veins.

Many invertebrates, including crustaceans like the crayfish, have neurogenic hearts. The myocardial cells will not beat without neural input from the cardiac ganglion. The resting heart rate and contractile force are set by this neural input. Crayfish also have an open circulatory system. Blood flows into the heart through dorsal ostia, and is pumped into body sinuses through arteries at both the posterior and anterior ends of the ventricle. The beats of both myogenic and neurogenic hearts are modulated by neurotransmitters. Vertebrate hearts are excited by epinephrine and inhibited by acetylcholine. In living crayfish, cardioexcitatory peptides increase the heart's rate and contractile force, while GABA inhibits both rate and force. Biogenic amines like Serotonin and Dopamine also have effects on the crayfish heartbeat. In this laboratory exercise, students will use a force transducer to monitor the contractility of the crayfish heart as it is subjected to various imposed conditions, such as: the effect of adding Serotonin and GABA to change the heart rate and contractile force of the exposed heart; and the effect of cold temperature on cardiac muscle activity.

The Dissection

1. Cover a crayfish with ice for 5 minutes.

2. With scissors, remove the claws, walking legs, and abdomen ("tail") from the crayfish. Collect the hemolymph for the blood smear.

3. The crayfish heart is located dorsally, at the posterior end of

the thorax. Use the scissors to carefully remove a small section of carapace over the heart. The hypodermis, the red membrane directly beneath the carapace, should also be removed.

4. The beating yellow-white ventricle of the heart should be clearly visible.

5. Use four #2 insect pins to pin the crayfish securely into a wax or Sylgard dish.

6. Cover the crayfish with 100 ml of room temperature Ringer's. If 100 ml of saline doesn't cover the crayfish, use more. It is important to keep track of exactly how much saline is in the dish.

The Preparation

1. Place the dissection dish so that the crayfish ventricle is directly below the end of the

transducer. The force transducer should be about 15 cm above the heart, with the blade of the transducer being horizontal (Figure AM-7-L1).

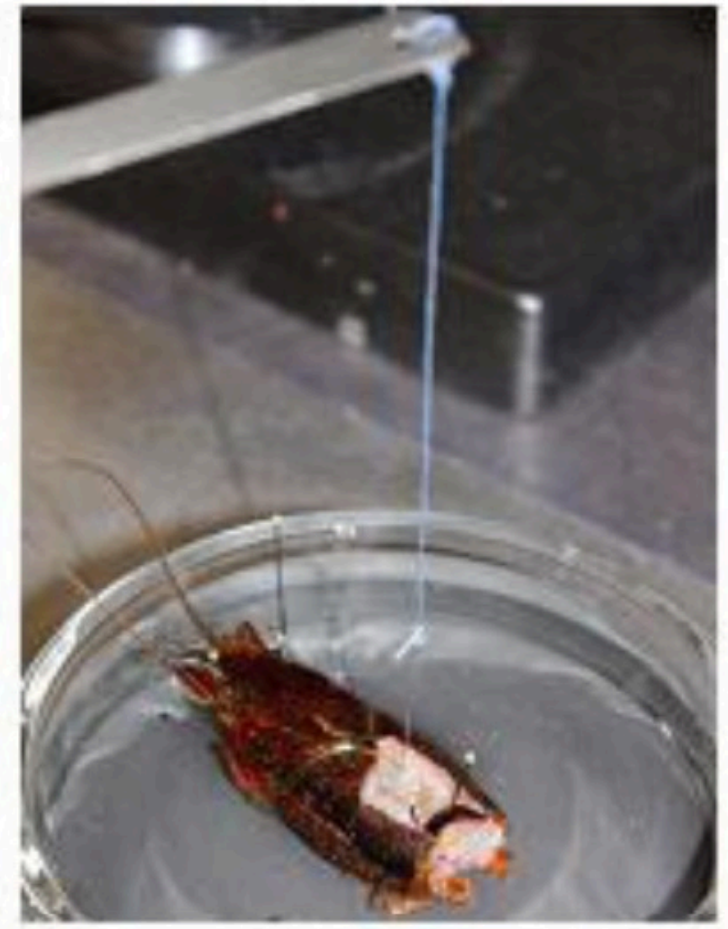


Figure AM-7-L1: *The crayfish heart preparation*

2. Bend a metal pin to form a hook. Tie a 20 cm length of thread behind the head of the hook.

3. Push the hook through the ventricle wall until the bend of the hook is inside the heart.

4. Tie the loose end of the thread to the hole in the blade or on the hook of the transducer. Loosen the clamp holding the transducer

and gently raise it on the ring stand. Put enough tension on the thread to raise the ventricle very slightly. (Figure AM-7-L2).

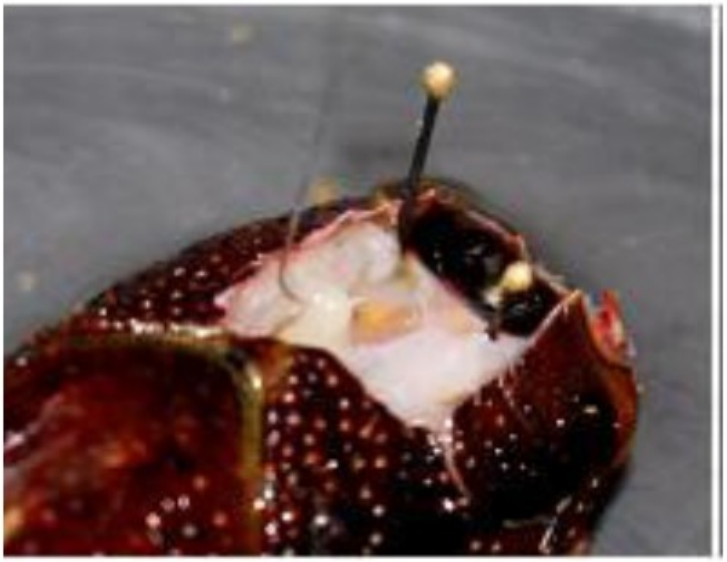


Figure AM-7-L2: Force transducer hook placed through the ventricle of the heart.

Warning: The heart preparation used in this experiment is functional for a limited period of time. Keep the heart muscle covered in saline. To conserve time, complete all the exercises in the experiment before analyzing the data.

Exercise 1: The Heart Rate

Aim: To record the mechanical trace produced by the contraction of a resting heart, and to determine the resting heart rate.

Approximate Time: 30 minutes (including dissection)

Procedure

1. Type Resting in the Mark box to the right of the Mark button.

2. Click the Record button and press the Enter key on the keyboard to attach the comment to the record. Click AutoScale to increase the size of the deflection on the Main window.

3. Record the heart contractions for thirty seconds. A sample recording can be seen in Figure AM- 7-S3.

4. Click Stop to halt the recording.

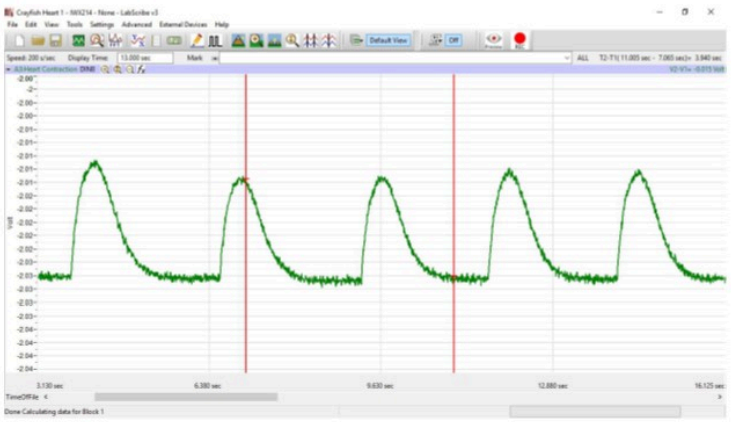


Figure AM-7-S3: *Recording of the contractions of the crayfish heart.*

5. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Designate the file type as *.iwxdata. Click on the Save button to save the data file.

Exercise 2: Effects of Cold Temperature

Aim: To record changes in heart rate after the heart is bathed in cold Ringer's solution.

Approximate Time: 15 minutes

Procedure

1. Type Room Temp Ringer's in the Mark box to the right of the Mark button.

2. Click the Record button and press the Enter key to attach the comment to the recording. Click AutoScale to increase the size of the deflection on the Main window.

3. Record the heart contractions for thirty seconds.

4. Click Stop to halt the recording.

5. Draw off the room temperature Ringer's with a syringe, and add chilled saline, taking care to replace the saline with the same volume as was drawn off.

***Warning*: It is important to keep the saline at the same depth throughout the experiment, as the depth of the saline will affect the amplitude of the recorded beats. The dissecting dish should also be firmly affixed to the table with clay so that it won't be accidentally bumped, changing the tension on the ventricle.**

6. Type Cold Ringer's in the Mark box.

7. Click the Record button and press the Enter key to attach the comment to the recording.

8. Record until the heart has recovered from the effects of cold Ringer's solution.

Note: Recovery is when the amplitude and rate of the heart contraction have returned to the resting values.

9. Click Stop to halt the recording.

10. Select Save in the File menu.

Exercise 3: Effects of Drugs

Aim: To monitor the effects of Serotonin and GABA on the amplitude and rate of heart contraction. Approximate Time: 45 minutes

Procedure-Serotonin

1. Type Pre-Serotonin control in the Mark box to the right of the Mark button.

2. Click the Record button. Press the Enter key on the keyboard to mark the recording. Click AutoScale to increase the size of the deflection on the Main window.

3. Record the heart contractions for thirty seconds.

4. Type Serotonin 10^{-6}M in the Mark box to the right of the Mark button.

5. Add 100 microliters of the 10^{-3}M stock Serotonin solution to the saline in the dish. Gently stir the saline to disperse the Serotonin.

Note: If you used a volume other than 100 ml, adjust the amounts of the stock solution accordingly. For example, if you covered the crayfish with 150 ml, add 150 microliters of the stock Serotonin solution to create a 10^{-6}M Serotonin solution.

6. Click Record to start the recording, and press the Enter key on the keyboard.

7. Record the effects of 10^{-6}M Serotonin for two minutes.

8. Click Stop to halt the recording.

9. Add an additional 900 microliters of the 10^{-3}M Serotonin solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the Serotonin throughout the saline.

10. Type Serotonin 10^{-5}M in the Mark box to the right of the Mark button.

11. Click Record to start recording and press the Enter key.

12. Record for two minutes.

13. Click Stop to halt the recording.

14. Add 10 ml of the 10^{-3}M Serotonin stock solution to the saline in the dish to create a 10^{-4}M Serotonin solution.

15. Type Serotonin 10^{-4}M in the Mark box to the right of the Mark button.

16. Click Record to start recording and press the Enter key to attach the comment to the recording.

17. Record for two minutes.

18. Click Stop to halt the recording.

19. Replace the saline with 100 ml of fresh saline, and allow the heart to recover.

Note: It is possible that the heart will not return to pre-treatment conditions. In this case, wait until the heart has come to a new steady-state, and record the heartbeat at that time. This should occur within ten minutes after the saline change.

20. Type Post-Serotonin recovery in the Mark box.

21. Once the heart has recovered, click Record to start the recording and press the Enter key.

22. Record for thirty seconds.

23. Click Stop to halt the recording.

24. Select Save in the File menu.

Procedure-GABA

1. Type Pre-GABA control in the Mark box to the right of the Mark button.

2. Click Record to start the recording and press the Enter key to attach the comment to the recording.

3. Record for thirty seconds.

4. Click Stop to halt the recording.

5. Add 100 microliters of the 10^{-3}M stock GABA solution to the saline in the dish. Gently stir the saline to disperse the GABA throughout the saline.

6. Type GABA 10^{-6}M in the Mark box to the right of the Mark button.

7. Click the Record button and press the Enter key.

8. Record the heart contractions for two minutes.

9. Click Stop to halt the recording.

10. Add an additional 900 microliters of the 10^{-3}M GABA solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the GABA throughout the saline.

11. Type GABA 10^{-5}M in the Mark box to the right of the Mark button.

12. Click Record to start recording and press the Enter key.

13. Record for two minutes.

14. Click Stop to halt the recording.

15. Add 10 ml of the 10^{-3}M GABA stock solution to the saline in the dish to create a 10^{-4}M GABA solution. Gently stir the saline to disperse the GABA.

16. Type GABA 10^{-4}M in the Mark box to the right of the Mark button.

17. Click Record to start recording and press the Enter key to attach the comment to the recording.

18. Record for two minutes.

19. Click Stop to halt the recording.

20. Replace the saline with 100 ml of fresh saline, and allow the heart to recover.

21. Type Post-GABA recovery in the Mark box.

22. Once the heart has recovered, click Record to start the recording and press the Enter key.

23. Record for thirty seconds.

24. Click Stop to halt the recording.

25. Select Save in the File menu.

Record your data in the data tables for each exercise in the lab report. Make sure to answer the questions at the end of the lab report as well.

Lab 7: Defense Mechanism

Materials needed for bee venom activity:

Nitrile gloves
Melittin from bee venom (> 85% purity) in a 1 mg/ml stock solution (sterile water)
Human cancer cell line in 96-well plates that are ready to use with 100 ul of cell media
Human control cell line that are ready to use
Cell medium for each cell line
Pipettors (20 ul, 100 ul, and 1000 ul)
Filter pipette tips
Ice bucket filled with ice (1 per group)
Inverted microscope
Sterile water (5 ml)
Four 1.5 ml microcentrifuge tubes in a rack
Sharpie marker for labeling
CellTiter-Glo (CTG) 2.0 Reagent (light sensitive)
Cell counter

Background

Melittin is the main component of honeybee venom, responsible for the pain from a sting by destroying cell membranes. It has a wide range of biological and pharmacological effects, including significant potential as an antibacterial agent and, when modified, as an anticancer drug. Research is exploring its therapeutic potential for conditions like cancer and chemotherapy-induced pain.

- **Venom component**: Melittin makes up about 52% of the dry mass of honeybee venom and is the primary cause of pain associated with a sting.
- **Cell membrane disruption**: Its most significant toxicological effect is the destruction of the cell membrane, which is active against a wide range of animal cells.
- **Antibacterial properties**: Melittin is also produced by the bee itself in its fat body and acts as an antibacterial agent. It is particularly effective against bacteria like E. coli and Listeria monocytogenes.
- **Therapeutic potential**: Researchers are investigating melittin's potential for medical use due to its ability to induce cell death and other effects.
- **Anticancer**: It has shown potent effects against certain aggressive breast cancer cells and can be combined with chemotherapy to suppress tumor growth.
- **Pain and inflammation**: It may also be used to treat

inflammation and pain, such as chemotherapy-induced peripheral neuropathy.
- **Challenges**: A challenge for human use is its non-specific toxicity and hemolytic activity, which researchers are trying to overcome by creating modified versions of melittin that target specific cells.

Therefore, as an application of this defense mechanism, the main aim of this lab will be to determine if there is a specific dose of melittin that can selectively destroy significantly more cancer cells than non-cancer cells.

Procedure

Step 1: Make serial dilutions of the 1 mg/ml melittin stock solution to produce three new solutions with 100 µg/ml, 5 µg/ml, 0.5 µg/ml, and 0.05 µg/ml and 0 µg/ml concentration of melittin in the respective cell medium for each cell line with a final volume of 1 ml. For the new concentrations make this in a new labeled 1.5 ml microcentrifuge tube. Invert the tubes to mix the solutions and place these on ice for the time being.

Note: show me your dilution calculations and what you plan to do before you start pipetting

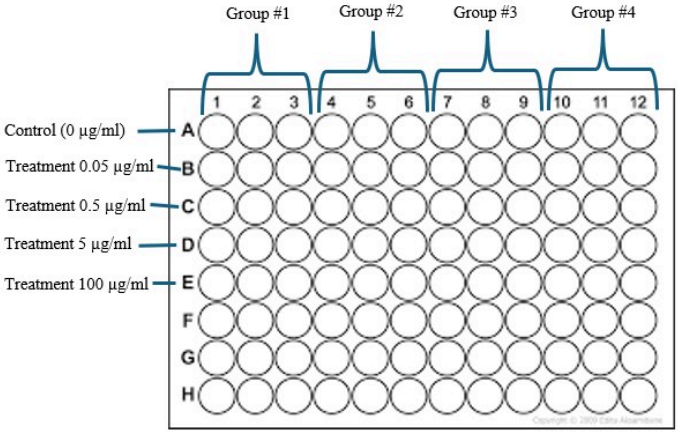
Step 2. Identify the morphology and count the number of cells underneath the inverted microscope for each well your lab group is assigned to for the cancer and control cell line. Record your morphological observations and number of viable cells in your lab report.

Step 3. **Refer to the plate layout at the front of them room for which wells have which concentrations to be added to them.** Using a pipettor, slowly remove 100 µl of the cell media from each well you will be treating and add 100 µl of the melittin concentrations you made to the respective well, do this 3 times for the control and each treatment and incubate at room temperature for 1 hr. Make

sure your wells are labeled and your group initials are indicated on top of the plate.

Step 4. To determine viability, add 100 µl and incubate cells with CellTiter-Glo (CTG) 2.0 Reagent for 10 min. Quantify cell viability using a visual inspection based on the amount of fluorescence underneath the inverted microscope and a cell counter. Record your observations and the number of viable cells for all of your wells. The more fluorescence, the more viable the cells are.

Step 5. Compile the data as a class in an excel sheet and conduct a Kruskal Wallis test to determine if there are any significant differences across the treatment groups. Run a Wilcoxon rank sum test across all the pair-wise comparisons if you find a significant difference with the Kruskal Wallis test. Make a dose dependent response curve across the treatment concentrations with a log scale. Write a results statement and a concluding paragraph as part of your lab report.



***Note: Two of these plates will need to be made as a class, one for the control cell line and one for the cancerous cell line.**

Lab 10: Spirometry

Materials needed and setup

EQUIPMENT

The Schiller ultrasonic spirometry (SpiroScout) system is a combined ECG-spirometry system that can be selected for cardiovascular or pulmonary function simply by changing the peripheral hardware that is attached to the main unit (AT-102) via the patient cable.

The Schiller spirometry system is an open pneumotacho-pulmonary measuring system that allows thorough cleaning of the components between subjects. By using one time use disposable Scout tubes (mouthpiece), potential HIV (AIDS) or TB infection is prevented. **SO BE SURE TO USE ALCOHOL TO CLEAN SHARED EQUIPMENT.**

IMPORTANT: Before starting the experiment the subject should practice breathing through the mouth piece with nose clips on so that he or she becomes familiar with this type of breathing. The subject should be in a standing position.

Background

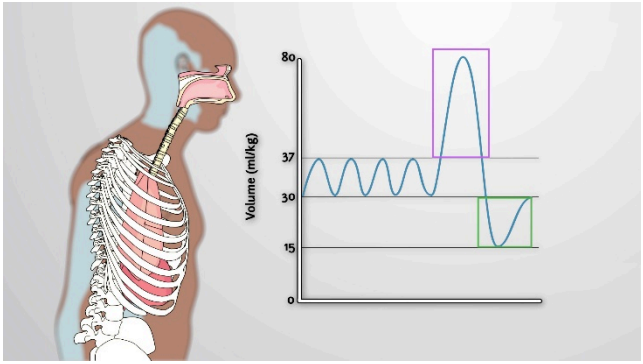
Human lung capacity is the total amount of air the lungs can hold, which is about 6 liters for an adult male and varies based on age, gender, and body composition. It is measured through spirometry and includes various lung volumes like tidal volume (air from normal breathing), inspiratory reserve volume (additional air inhaled), expiratory reserve volume (additional air exhaled), and residual volume (air left in lungs after exhalation).

Factors affecting lung capacity

- **Age**: Lung function naturally declines after about age 35.
- **Gender:** Men typically have a larger lung capacity than women.
- **Body size and composition**: Factors like height and weight influence lung capacity.
- **Altitude**: Living at high altitudes can lead to a larger lung

capacity.

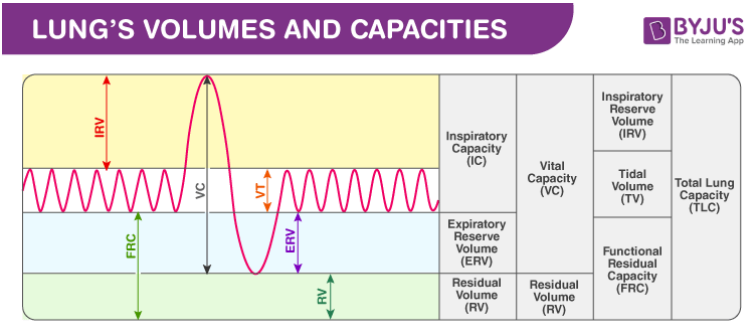
- **Respiratory diseases**: Conditions like asthma or COPD can change lung volumes.



ABBREVIATIONS AND DEFINITIONS

RR	Respiration Rate	The number of breaths per minute
MV	Minute Ventilation	The volume of air moved in one minute
TV	Tidal Volume	The volume of air inspired or expired during a breath when individual is at rest.
ERV	Expiratory Reserve Volume	The volume of air that can be expired after a normal expiration.
IRV	Inspiratory Reserve Volume	The volume of air that can be inspired after a normal inspiration.
VC	Vital Capacity	Maximum amount of air that can be moved in one exhalation. VC=TV+IRV+ERV
TLC	Total Lung Capacity	Maximum amount of air that can be in the lungs.
SVC	Slow Vital Capacity	The volume of air that can be expired following a deep inspiration.
FVC	Forced Vital Capacity	The volume of gas expired by the quickest possible exhalation after a maximal inhalation.
FEV_1	Forced Expiratory Volume in one second	The volume of air moved during the first second of forced expiration. This is the first second of FVC.
MVV	Maximum Voluntary Ventilation	The maximum volume of air that can be moved on expiration while breathing as deeply and as rapidly as possible.
RV	Residual Volume	Volume of air always present in the lungs

AVR	Alveolar Ventilation Rate	Amount of air that reaches the alveoli and is available for gas exchange. Anatomic dead space where air that moves in and out of the conducting tubes and never reaches the alveoli.



Practice: LOOK AT THE GRAPH ABOVE AND QUANTIFY ALL THE LUNG VOLUMES.

In this laboratory exercise you will measure your lung volumes and various capacities by spirometry. The ability of a patient to ventilate the lungs in a given time period is an excellent indicator of the functional capacity of the lungs.

Procedure:

1. Setting up the SpiroScout Ultrasonic Spirometry System

a. Plug in the power cable into the back of the Cardiovit AT-102 System. Make sure that the power I/O light is on.

b. Insert the plug of the spirometry flow sensor into the socket marked RS 232–Flow Sensor on the right hand side of the unit and tighten the two securing screws. CAUTION: The flow sensor SpiroScout contains a sensitive measuring device and must always be handled with care and not be dropped or subjected to any sudden blows.

2. Press the **ON** button on the keyboard.

3. Insert the ScoutTube. The ScoutTube (the disposable mouthpiece) can only be positioned in one direction and no force is necessary to insert it. Align the arrow on the ScoutTube with the

notch in the SpiroScout and push until fully home. The ring of the tube should be recessed in.

4. In order to select pulmonary function testing, you need to enter the patient data first: Press **PATIENT DATA**. Type in data using the keyboard. Movement from one data field to another is accomplished by using the **ENTER** key (or Down Arrow key ▼). Wrongly typed characters can be deleted with the **DELETE** key. Note: If a new patient name is entered (or the machine is turned off), all the previous patient's data are automatically deleted.

Input of patient data:

a. Patient number: OMIT
b. Last name:
c. First name:
d. Born (Date of birth):
e. Age: Cardiovit will calculate this.
f. Sex:
g. Height in inches:
h. Weight in lbs.:
i. Blood pressure in mm Hg (7 figures max) – e.g. 120/80
j. Ethnic: C/H/B/A/M (Causasian /Hispanic /Black /Asian / Mexican)
k. Medication: OMIT.
l. Ref-No.: OMIT
m. Pacemaker: Yes/No

5. Once all patient data has been entered, select **MENU**.

6. To enter the pulmonary testing mode, press **SPIRO** (you may have to select **MENU** a second time to find this option).

The following describes the procedures needed to perform the typical spirometry tests which are performed in order to assess pulmonary function.

Each student will calibrate the spirometry unit and perform the pulmonary function tests described below. Repeat each measurement so that **good readings are obtained three times for each procedure** (three readings are required to meet American Society of Thoracic Medicine standards.) **NOTE: At the end of each**

test press stop in order to let the system know that you have finished. If any one of your three readings appears too large or small, ask your instructor for assistance in deleting it.

1. To measure **Minute ventilation (MV)**, Select **MVV**—Maximum Voluntary Ventilation. The subject puts on a nose clip. The subject stands and places the mouthpiece in their mouth. Subject should exhale before starting the measurement. The computer operator hits **RUN TEST/ START**. When the "Ready for Measurement" message appears on the screen the subject **breathes normally for 12 seconds**, without looking at the screen. When the subject has the mouthpiece in their mouth the computer operator will hit **RUN TEST/ STAR**T for each test. **THE LIPS MUST FORM A TIGHT SEAL AROUND THE MOUTHPIECE!** You do not need to hit STOP. The test automatically stops after 12 seconds. To begin the next test, simple press **RUN TEST/ START** again. When all three tests are completed, press **REVIEW/ PRINT**, then select **PRINT** and then press **PRINT MEAS1**. The report will show you the values of Maximum Voluntary Ventilation (MVV), Respiratory Rate (RR), and Tidal Volume (TV). To calculate Minute Ventilation, multiply RR x TV._____L/min

2. Press **RESUME** and then press **TEST**, and then press **SVC (Slow vital capacity)**. Read the directions on the screen. The subject stands and places the mouthpiece in their mouth. The computer operator will hit **RUN TEST/ START**. When the "Ready for Measurement" message appears on the screen the subject will take three normal breaths and then perform a vital capacity reading by taking a maximum inhalation followed immediately by the biggest exhalation possible. The computer operator will press **END TEST/ STOP** after the exhalation. **Repeat this test two more times** by pressing **RUN TEST/ START** each time. When the tests are completed the computer operator will hit **REVIEW/ PRINT**, then select **PRINT** and then press **PRINT MEAS1**.

3. Press **RESUME** and then press **TEST**, and select **FVC (Forced vital capacity)**. Read the directions on the screen. The computer operator will hit **RUN TEST/ START**. When the "Ready for

Measurement" message appears on the screen the subject will take the deepest inspiration, put the mouthpiece in her mouth and give the strongest, **fastest** expiration possible and at the end of this expiration take one full inspiration before the test is ended. **Repeat this test two more times.** Press **RUN TEST/ START** to begin each new test. When the tests are completed the computer operator will hit **REVIEW/ PRINT**, then select **PRINT** and then press **PRINT MEAS1**.

4. Press **RESUME** and then press **TEST**, and select **MVV (maximum voluntary ventilation)**. The subject inserts the mouthpiece. The computer operator will hit **RUN TEST/ START.** When the "Ready for Measurement" message appears, the subject will breathe in and out as fast and as hard as possible for 12 seconds. The computer will stop the test once 12 seconds has been reached. Have the patient sit down after the test as they will be lightheaded. When the tests are completed the computer operator will hit **REVIEW/ PRINT**, then select **PRINT** and then press **PRINT MEAS1**.

5. To turn off Cardiovit 102, press the **OFF** key.

Lab 11: Animal Fluid Balance: Osmoregulation

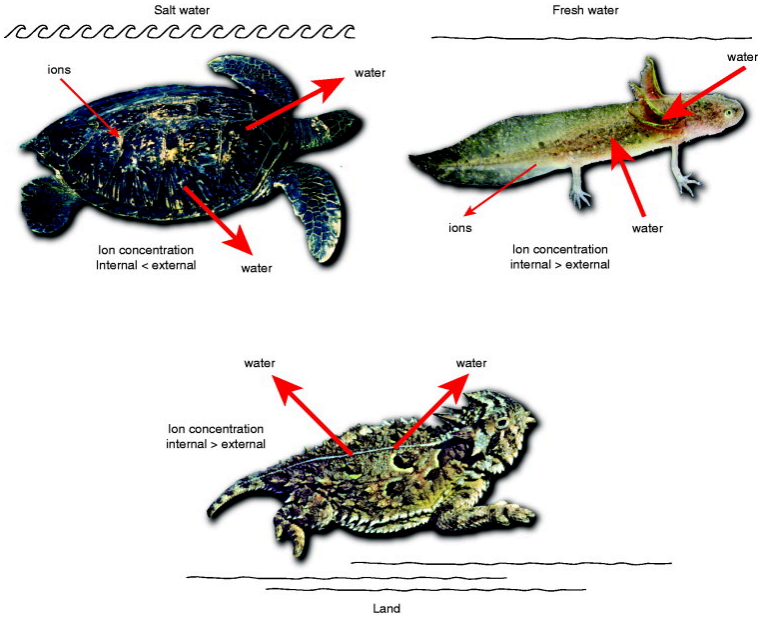
Overview

For the biochemical reactions in cells to proceed properly, the optimal conditions of the internal environment of the cells must be maintained. The cell membrane forms the barrier that separates the internal environment of the cell from the external environment. The membrane maintains the balance of fluid gained and lost by the cell so that any fluctuations in the osmotic and ionic conditions in the cell are minimized, and reactions can proceed normally.

Animals living in the ocean are exposed to an external environment that has minimal fluctuations in osmotic and ionic conditions. These relatively constant conditions in the external environment, coupled with the similarity of the internal environment to the chemical composition of seawater, make the maintenance of the internal environment of these animals relatively simple.

Animals living in freshwater are exposed to an environment with low concentrations of ions and a high concentration of water. In this type of environment, cells lose ions and take on water. Animals have developed mechanisms that remove water and retain ions to prevent cells from swelling and bursting.

Conversely, animals living in air are exposed to a very harsh environment with a low concentration of water. Without proper levels of water, cells can dehydrate and shrink. In this environment, animals have developed mechanisms that conserve water and remove excess ions.

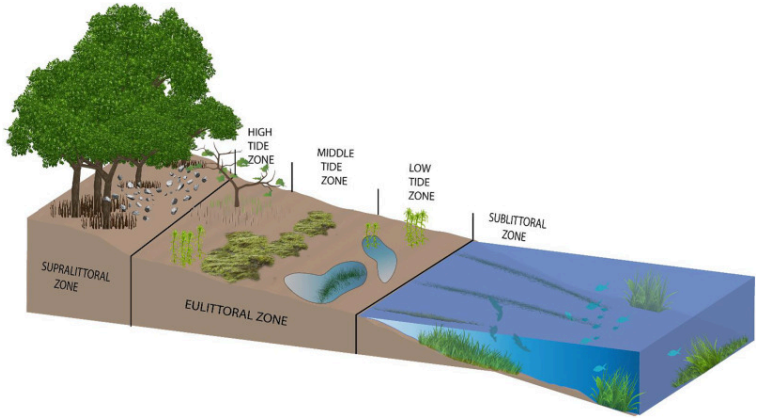


The pioneer French physiologist Claude Bernard described the internal fluid of plasma and extracellular fluid that bathes most of the cells in our bodies as the *milieu interieur*. In order to maintain this solution at near constant conditions, organisms use many different processes, which are encompassed by the term *homeostasis*. In most cases, healthy organisms use a negative feedback mechanism that quickly detects any changes in their internal conditions and corrects the internal environment before drastic changes to their cells occur.

Background

Sodium is the predominant cation in the extracellular fluid of multicellular animals. The high level of sodium ions in seawater results in minimal osmotic stress in many marine organisms. In these animals, therefore, minimal ionic and osmotic regulation is required. Furthermore, the large volume of water in the ocean ensures minimal fluctuations of the osmotic environment.

Some marine organisms do not spend all of their time in the ocean. Some live in the intertidal region where they experience periods of drought, while others may live in tidal pools. In the latter case, the relatively small volume of water in the tidal pool may result in fluctuations in the osmotic environment. The level of sodium in the pool may increase when the sun evaporates water or decrease when freshwater is added through rain or a river. Thus, animals that are trapped in a tidal pool must be able to adapt to short-term osmotic stress and survive for about 12 hours until the next tide.



In this laboratory, you will use a series of dilutions of seawater (with deionized water) to measure the effects of solute concentration on the movement of water into or out of an aquatic worm or slug. You will place a worm in each solution and then measure its weight change every 10 minutes for one hour.

Learning Goals for this Experiment:

1. Students will weigh and observe polychaete worms in different marine salinity dilutions.
2. Students will understand the correlation between saline concentration and osmoregulation in marine organisms.
3. Students will determine the iso- hypo- and hyper-tonic

environments based on the loss or gain of weight due to osmosis over time.

Equipment Required

Computer
IWX/214, power supply and USB cable
FT-104 Force Transducer
Ring stand and clamp
Weigh boat and pennies (each weighs about 3 g)
5 x 250 ml beakers per group
2 x 100 ml graduated cylinders per group
Forceps
Marine Worms
Artificial Seawater (see Appendix)

Start the Software

1. Click on LabScribe
2. Click Settings Animal Fluid Balance Osmoregulation

The following should be already set up for you:

1. The force transducer will be plugged into Channel 3 input of the IWX/214 (Figure FB-1).
2. The transducer will be attached to a ring stand using a 90˚ clamp, so that the transducer is horizontal.
3. A weigh boat will be attached to the end of the transducer arm via thread (Figure FB-2).

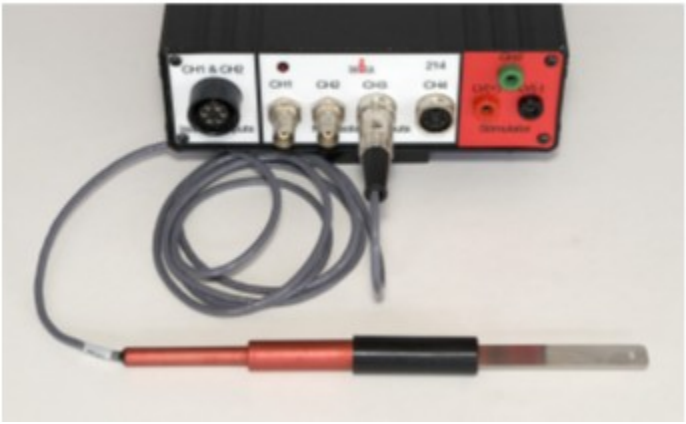


Figure FB-1: The force transducer is connected to Channel input of the IWX/214.

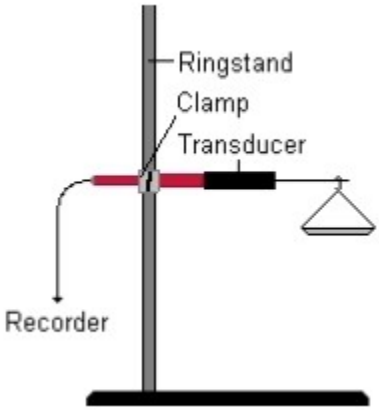


Figure FB-2: Equipment setup to record weight placed in a weigh boat.

Experiment FB-1: Osmoregulation

Calibration of FT-104 Force Transducer

Aim: To calibrate the force transducer used to measure weight gain or loss of the animal.

Approximate Time: 15 minutes

Procedure

1. Turn on the iWorx box.

2. Type "**No Weight**" in the Mark box to the right of the Mark button. Click the Record button, and press the Mark button to the left of the mark box. Record for ten seconds with no weight hanging from the arm or hook of the transducer.

3. Count the number of pennies you have and multiply their number by three (the weight of a penny in grams).

4. Type the **weight of the pennies** in the Mark box. Place the pennies on the weight pan and press the Mark button. Click the AutoScale button next to the channel title area. Record for ten more seconds (Figure FB-3).

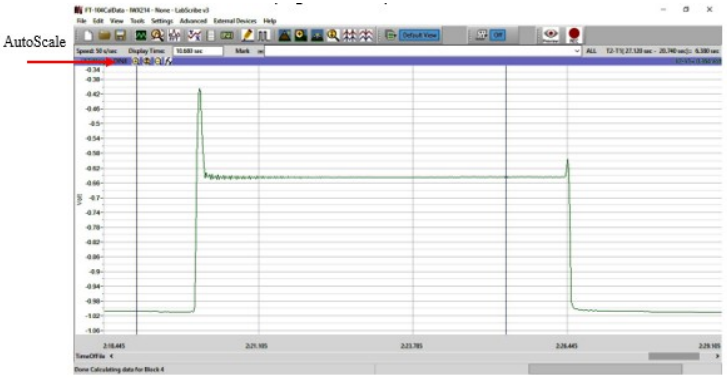


Figure FB-3: Recording of different known weights used to calibrate FT-104 force transducer.

5. Click Stop to halt the recording.

6. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

7. Remove the pennies from the weight pan.

Units Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.

2. Use the Display Time icons on the LabScribe toolbar (Figure FB-4) to adjust the Display Time of the Main window to show the complete calibration data on the Main window. The required data can also be selected by:

• Placing the cursors on either side of data required.

• Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the complete calibration data to the width of the Main window.

3. Click the Double Cursor icon so that two cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the FT-104, and the second cursor on the flat section of data collected when the pennies were attached to the transducer.

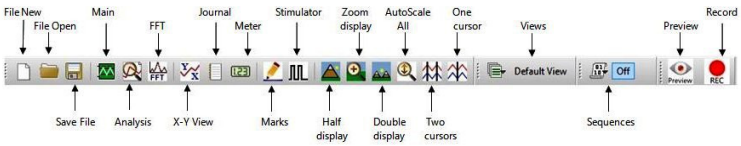
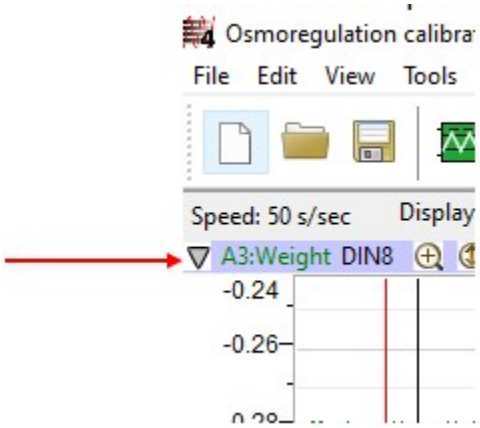


Figure FB-4: The LabScribe toolbar

4. To convert the output of the force transducer from a voltage to the grams of force:

• Click on the arrow next to the title of the Weight channel to open the channel menu.
• Select Units from the channel menu and Simple from the submenu.



5. The Simple Units Calibration window will appear (Figure FB-5). On this window:
• Select 2 point calibration from the pull-down menu in the upper-left corner of the window.
• Put a check mark in the box next to Apply units to all blocks.
• Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
• Enter zero in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter the weight of the pennies in the box to the right of the corresponding voltage recorded when the weight of the pennies was hung on the hook of the transducer.
• Enter the name of the units, grams, in box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

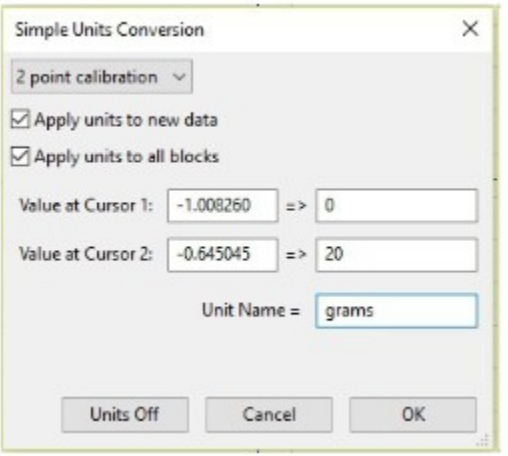


Figure FB-5: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the weight used in calibration.

Practice Weighing Technique

Aim: To develop a consistent technique of weighing a worm.

Approximate Time: 15 minutes

Procedure

1. Use forceps to remove a worm from seawater. Blot the worm with paper towels to remove excess water.

2. Type No Worm in the Mark box to the right of the Mark button.

3. Click the Record button to record a baseline of ten seconds while only the weight pan is attached to the transducer. Continue recording.

4. Type **Worm** in the Mark box and mark the recording. Place the worm on the weight pan attached to the transducer.

5. Continue to record for ten seconds after the worm was placed on the weight pan. Click Stop to halt the recording.

6. Replace the worm in the seawater.

7. Repeat Steps 1 through 6 for two other worms.

Data Analysis

1. Scroll through the data file to the weighing of the first worm.

2. Use the Display Time icons to adjust the Display Time of the Main window so that the output of the transducer before and after the worm was placed on the weight pan is displayed on the Main window.

3. Data can be collected from the Main window or the Analysis. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.

4. The mathematical functions, V2-V1 and T2-T1, should be showing. The values for these parameters are displayed on screen.

5. Maximize the height of the trace on the Weight Channel by clicking AutoScale All from the toolbar.

6. Place the cursors on the recording before and after the addition of the worm to the weight pan. The value for V2-V1 on the Weight channel is the weight of the worm.

7. The weight of the worms can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal.

8. The functions in the channel pull-down menus of the Analysis window can also be used to enter the name and value for V2-V1 from the recording to the Journal. To use these functions:

• Place the cursors at the locations used to measure the weight of the worm.

• Transfer the name of the mathematical function used to determine the weight to the Journal using the Add Title to Journal function in

the Weight Channel pull-down menu.
• Transfer the weight to the Journal using the Add Ch. Data to Journal function in the Weight Channel pull-down menu.

9. Repeat Steps 2 through 8 to find the weights of the other two worms weighed in this exercise. Record the weights in the Journal and on Table FB-1.

10. Select Save in the File menu.

Osmoregulation

Aim: To measure changes in the weights of five (5) worms placed in different osmotic environments.

Approximate Time: 30 minutes

Procedure

1. Use forceps to remove a worm from seawater. Blot the worm with paper towels to remove excess water.

2. Type **No Worm** in the Mark box to the right of the Mark button.

3. Click the Record button to record a baseline of ten seconds while only the weight pan is attached to the transducer. Continue recording.

4. Type **Worm 1** in the Mark box. Place the worm on the weight pan attached to the transducer. Press the mark button to mark the recording.

5. Continue to record for ten seconds after the worm was placed on the weight pan. Click Stop to halt the recording. Note the time when the worm was weighed.

6. Record the weight of the worm in the Journal and on Table FB-2.

7. Place the worm in the 100% seawater (or appropriate environment) solution.

8. Repeat Steps 1 through 6 for each of the four other worms used in this exercise. Each worm goes into a different solution: 90, 80, 70, or 60% seawater.

9. Every ten minutes, remove each worm from its solution. Blot the worm, weigh it, and return it to the same solution.

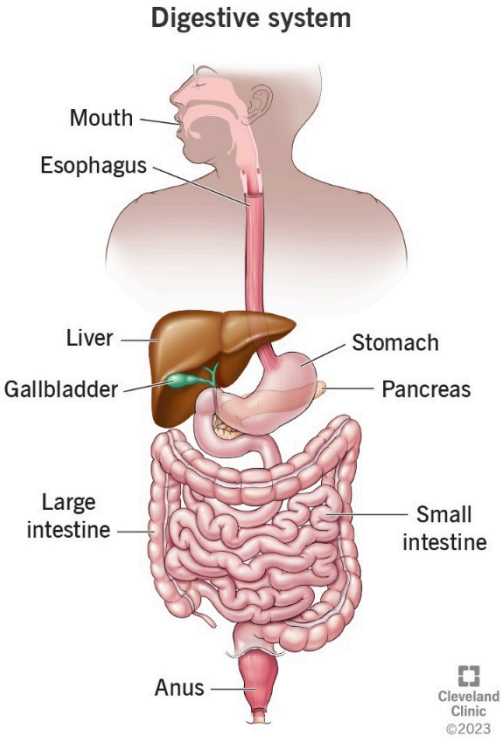
10. Record the weight of the worm in the Journal and on Table FB-2.

11. Weigh all the worms until you have seven weight values for each worm.

Lab 12: Metabolism and Digestive Physiology

Background

Digestion is the process of breaking down food into smaller molecules that the body can absorb for energy, growth, and repair. This is accomplished through two main types of digestion: mechanical, which involves physical breakdown like chewing, and chemical, which uses enzymes and acids to break down food. The process starts in the mouth and continues through the esophagus, stomach, and intestines, with the help of organs like the liver and pancreas.



Key organs involved

Mouth: The start of digestion, where food is mechanically broken down by chewing and chemically broken down by saliva.

Esophagus: A tube that carries food from the mouth to the stomach.

Stomach: A muscular organ that mixes food with digestive juices and acids to break it down further.

Small intestine: The primary site for nutrient absorption and further chemical digestion.

Large intestine: Absorbs water from the remaining indigestible food matter.

Liver and pancreas: These organs produce enzymes and other substances (like bile) that are crucial for chemical digestion in the small intestine.

Objectives

1. To list the digestive system enzymes involved in the digestion of proteins, fats, and carbohydrates; to state their site of origin; and to summarize the environmental conditions promoting their optimal functioning.
2. To recognize the variation between different types of enzyme assays.
3. To name the end products of digestion of proteins, fats, and carbohydrates.
4. To perform the appropriate chemical tests to determine if digestion of a particular foodstuff has occurred.
5. To cite the function(s) of bile in the digestive process.
6. To discuss the possible role of temperature and pH in the regulation of enzyme activity.

Salivary Digestion of Carbohydrates

Digestion of Carbohydrates begins in mouth where the salivary glands secrete an enzyme, amylase that begins the hydrolysis of complex polysaccharides:

Plant starches or animal glycogen ——— >Disaccharides (Maltose, sucrose, lactose)

Salivary amylase has an optimum pH of around 6.8, which is roughly pH found in the mouth.

In the following experiment, we will examine amylase digestion of starch, using Benedict's test to measure maltose formation and Lugol's (iodine) solution to test for starch.

Experimental Procedure:

1. Collect 10 ml of your own saliva in a graduate cylinder. Dilute saliva with an equal amount of water if you are unable to collect 10 ml. (minimum of 7 ml of saliva is needed)
2. Test the saliva with pH paper. Is it acid or alkaline?
3. Prepare and label four test tubes as follows (Use 0.5% starch solution):

Tube 1:	3 ml. of starch + 3ml. of water.	Incubate at 37 C.
Tube 2:	3 ml. of starch + 3ml. of saliva.	Incubate at 37 C.
Tube 3:	3 ml. of starch + 3ml. of saliva.	Incubate on ice.
Tube 4:	3 ml. of starch + 3ml. of saliva + 5 drops of concentrated HCl.	Incubate at 37 C.

Incubate all test tubes for 1 hour.

4. **Starch Test**: Take 5 drops of incubate on a white tile and add 1 drop of Lugol's iodine. A dark purple color indicates presence of starch. Shades of reddish brown indicate lesser amount of starch. (+++), (++), (+) (-)

5. **Maltose Test:** Add 4 ml. of Benedict's solution and place in a boiling water bath for 2 mins. Compare the color developed. (+++) red; (++) orange; (+) green; (-) blue {PLACE IN THE SAME ORIGINAL TEST TUBE}

Gastric digestion of Proteins

Protein digestion begins in the stomach where the enzyme splits proteins into shorter polypeptide chains containing amino acids. Secretion and activation of pepsin occurs as follows: Chief cells in gastric pit ——> Pepsinogen + HCl —— >Pepsin

Experimental Procedure:

1. Place 3 ml. of albumin soln. (5%) in 4 test tubes.
2. Add the following solutions to the tubes:

Tube 1:	5 ml pepsin (5% solution) + 5 ml. HCl
Tube 2:	5 ml pepsin (5% solution) + 5 ml. water
Tube 3:	5 ml water + 5 ml. HCl
Tube 4:	5 ml pepsin (5.0 % solution) + 5 ml. NaOH (0.5 %)

3. Allow the tubes to incubate at 37 C for 1 hour. Test the final pH of the solution and estimate the amount of proteins digested using a scale of (+++), (++), (+), (-) to compare the four tubes.

Digestion of fat with pancreatic lipase and bile salts

Pancreatic lipase has a major role in fat digestion, but by itself lipase is ineffective, because it is a water-soluble enzyme trying to act on large lipid droplets, which are insoluble. Bile salts help overcome this problem by emulsifying fat into smaller droplets, so that lipase has a larger surface area for the hydrolysis of fats.

Pancreas also aids digestion by secreting sodium bicarbonate. This compound provides a pH around 7.8 in the small intestine, which is optimal for the action of pancreatin enzymes. In the following exercise, we will examine some aspects of the action of pancreatic lipase and bile salts on lipids.

Effects of Bile salt on Oil:

1. In each of two test tubes (A and B) place 3 ml of distilled water and 3 ml of vegetable oil.
2. To tube B add a small pinch of bile salts. Shake each tube for 30 secs. And observe it for several mins.

Effects of pancreatic enzymes:

3. Add litmus powder to dairy cream until a blue color is produced. Pre-incubate the litmus cream and a 1% pancreatin solution at 37 C for 5 mins.

The basis of this assay is a pH change that is detected by a litmus powder indicator. Alkaline or neutral solutions containing litmus are blue but will turn reddish in the presence of acid. Since fats are digested to fatty acids (organic acids) during hydrolysis, they

lower the pH of the sample they are in. Litmus cream (fresh cream providing the fat substrate to which litmus powder was added) will turn from a bluish color to pink if the solution is acid.

4. Prepare a series of tubes as follows:

Tube 1:	3 ml cream + 3 ml pancreatin
Tube 2:	3 ml cream + 3 ml water
Tube 3:	3 ml cream + 3 ml pancreatin + pinch of bile salts
Tube 4:	3 ml cream + 3 ml water + pinch of bile salts

5. Incubate all tubes in 37 C water bath for 1 hour or until a color change occurs in one tube. Blue litmus will turn pink in an acid environment. Test the pH using pH paper and note the odor of each tube.

DIGESTION

Carbohydrates:

Mouth:

Salivary Amylase

Polysaccharides ————————> Disaccarides, Trisaccharides and dextrins

Duodenum (Pancreatic juice):

Pancreatic Amyalse

Polysaccharides ————————————> Disaccarides and Trisaccharides

Intestine (Brush border Enzymes):

Dextrinase

dextrins ————————————> single dextrose (glucose)

Maltase

Maltose ———————————— >2 molecules of glucose

Sucrase

Sucrose ———————————— >glucose + fructose

Lactase

Lactose ————————————> glucose and galactose

Proteins:

Stomach:

Pepsin*

Proteins ————————————> Peptides

Duodenum (Pancreatic juice):

Pancreatic Trypsin^

Proteins ——————————————> Peptides

Chymotripsin@

Proteins ——————————————> Peptides

Carboxypeptidase

Peptides ——————————————– > removes –COOH group from peptide

Intestine:

Aminopeptidase

Peptides ——————————————– > removes amino (NH_2) group

Dipeptidase

Dipeptides ——————————————> Single amino acids

HCl

Pepsinogen (Inactive) ——————————————– >Pepsin

Enterokinase (from intestine)

^ Trypsinogen (Inactive) ——————————————– >Trypsin^

Trypsin

@Chymotrpsinogen (Inactive) ——————————————> Chymotrypsin@

Lipids:

Mouth:

Lingual Lipase

Triglycerides ——————————————> Fatty acids and monoglycerides

Intestine: (Bile and Pancreatic juice)

Bile Salts

Large Triglyceride mols. ———————————— >Broken into smaller mols.

Pancreatic Lipase

Triglycerides ——————————————> Fatty acids and monoglycerides

Hormones of GI tract

Hormone	Origin	Effects
1. Gastrin	Pyloric Stomach	1. Stimulates gastric secretion and its motility
2. Gastric Inhibitory Peptide (GIP)	Duodenal mucosa	1. Inhibits gastric secretion and its motility 2. Stimulates release of insulin
3. Secretin	Duodenal mucosa	1. Inhibits gastric secretion and its motility 2. Stimulates release of HCO_3 from pancreas 3. Increases rate of bile secretion
4. Cholecystokinin	Duodenal mucosa	1. Inhibits gastric secretion and its motility 2. Causes contraction of gall bladder 3. Stimulates secretion of pancreatic enzymes

Lab Report Guidelines

Your animal physiology laboratory report write up assignment:

From Lab 11: Osmoregulation, the goal will be to generate a lab report as if you were to submit a manuscript for publication in a peer reviewed journal. The formatting guidelines and expectations will correspond to this. The final draft to be submitted must include at least one scientific figure complete with a figure caption. You are required to properly report the statistics for the worm weight gain from the class dataset when comparing across the different saltwater concentrations. Qualitative observations should be reported as well in the results section. Methods should be reported detailed enough such that someone could repeat the experiment. In addition, the report should include at least three primary sources (peer reviewed journal articles only) other than your laboratory manual, the suggested reading, and your textbook. This paper has a 4-page limit on the main text (not including Abstract, Figures, Acknowledgements or Literature Cited). Papers should be double-spaced with 1-inch margins and be written in Times New Roman 12 pt font. Each report should contain an Abstract, Introduction, Materials and Methods, Results, Discussion, Figures, Acknowledgements, and Literature Cited section.

For more information on how to write up a lab report and to get additional guidance see the PDF file on Blackboard entitled: "How-To-Write-A-Lab-Report-Guide"

Sample Lab Report

Title: Testing the Biological Species Concept in *Drosophila melanogaster* and *Drosophila willistoni*

Abstract

The biological species concept claims that a species consists of a

population that can interbreed but that cannot breed with foreign populations. As a result of reproductive isolation, two populations that have been separated for a long time are less likely to mate than two species that have been separated for a short time. In this experiment, we compared changes in latency to copulation, singing, and the general morphology of *Drosophila willistoni* and *Drosophila melanogaster* populations from Peru (DMP) and Hawaii (DMH) in order to determine whether prezygotic barriers had developed between them and whether they could still be considered the same species according to the biological species concept. We hypothesized that the longer the populations of fruit flies had been separated from each other, the longer the latency to copulation and singing would be. Latency to copulation and to singing was recorded every minute for 30 minutes via scan sampling, and morphological observations were recorded before the experiment. We discovered that DMP and DMH fruit fly populations can be considered the same species and that *D. willistoni* would be considered a different species according to the biological species concept. In the future, it would be beneficial to ascertain the latency to copulation between male DMP and female DMH and *D. willistoni* flies as well as determine the extent to which morphological differences affect mate choice.

Introduction

According to the biological species concept, a species is a population that can interbreed but that cannot breed with other populations (Diamond, 2005). Speciation, or the independent evolution of two isolated populations into different species, results from reproductive isolation, which consists of postzygotic and prezygotic barriers that prevent gene flow between the populations (Nanda & Singh, 2012). Prezygotic barriers develop as mutations arise that give individuals a reproductive advantage by allowing them to distinguish between members of their own species and foreign ones (Sadava et al. 2014). Behavioral isolation, a barrier that involves species-specific signals in mating displays, facilitates recognition among viable mates of the same species (Nanda & Singh,

2012). For instance, fruit flies differentiate between each other through species-specific pre-copulatory behaviors like the vibration of a male fruit fly's wing when singing to a female (Iliadi et al. 2009; Nanda & Singh, 2012). Previous studies have found that reproductive barriers tend to increase the longer populations are separated (Coyne & Orr, 1997). Similarly, the biological species concept suggests that populations are less likely to breed the longer that they have been isolated from each other (Biology 2 Laboratory Manual, Swarthmore College, 2017).

In order to determine whether prezygotic barriers have developed between isolated populations of fruit flies and whether the populations have diverged as species, we mated populations of *Drosophila melanogaster* from Peru (DMP) and Hawaii (DMH) that have been separated for 150 to 10,000 years, as well as mated *Drosophila willistoni* that have been isolated for 35 million years (Keller, 2007; Biology 2 Laboratory Manual, Swarthmore College, 2017). We hypothesized that the longer the populations had been isolated from each other, the longer the latency to copulation and to singing would be.

Materials and Methods

QUANTITATIVE MEASUREMENTS AND ANALYSIS

Latency to copulation and to singing was recorded each minute via scan sampling. Ten female DMP flies were mated with ten male DMP, DMH, or *D. willistoni* flies per trial. Trials ended when flies copulated or when 30 minutes elapsed. A Kruskal-Wallis test assessed the differences in latency to copulation and singing between the DMH, DMP, and *D. willistoni* fruit flies. A Wilcoxin rank-sum test with a Bonferroni correction clarified these differences (Biology 2 Laboratory Manual, Swarthmore College, 2017).

QUALITATIVE MEASUREMENTS

Behavioral differences were observed through ad libitum sampling of two DMP flies and used to create an ethogram. Morphological differences between populations were also observed (Biology 2 Laboratory Manual, Swarthmore College, 2017).

Results

QUANTITATIVE RESULTS

Differences between the latency to copulation of *D. willistoni* fruit flies to DMH and DMP flies were statistically significant, as exhibited by the *D. willistoni* flies' lack of copulation (Fig. 1; x2 = 26.399, df=2, P<0.0001). A Bonferroni correction yielded a new critical value of 0.016. *D. willistoni* flies (median = 31 min; IQR = 0-0; count = 30) had a much greater latency to copulation than their DMP (median = 22.5 min; IQR = 8.5-13.5; count = 38, Z = 4.898190, P < 0.0001) and DMH (median = 17 min; IQR = 14-10.5; count = 37, Z = 4.822441, P < 0.0001) counterparts. The difference between the latency to copulation of DMP to DMH flies was not statistically significant, as displayed by their close proximity on the graph (Fig. 1; Z=0.259418, P=0.7953).

Differences between the latency to singing of the *D. willistoni* flies to DMH and DMP fruit flies were also statistically significant, as exhibited by the *D. willistoni* flies' higher placement on the graph (Fig. 2; x2 = 17.8614, df=2, P<0.0001). A Bonferroni correction yielded a critical value of 0.016. *D. willistoni* flies (median = 30 min; IQR = 1-25.25; count = 30) had a longer latency to singing than their DMP (median = 3 min; IQR = 8.25-1; count = 28, Z = 3.89453, P < 0.0001) and DMH (median = 5 min; IQR = 5.75-2.75; count = 32, Z = 3.36602, P = 0.0008) counterparts. The difference between the latency to copulation of DMP to DMH flies was not statistically significant, as displayed by their similarly short latencies (Fig. 2; Z=-0.69241, P=0.4887).

QUALITATIVE RESULTS

DMP flies had light yellow bellies and dark backs. One third of their tails were black, and their wings reflected a rainbow of light. They were the largest of all three fly species. Though smaller, DMH flies boasted a similar morphology to DMP flies. Half of their tails were black, and their wings reflected less light. Both DMP and DMH had dark red eyes and dark bands across their torsos. *D. willistoni*

flies were the smallest, lacked any black on their tails, were lightest in color, and did not have reflective wings.

Discussion

Our hypothesis that populations separated for a long time would be less likely to mate was supported. While DMP flies copulated within their own population and within the DMH population, *D. willistoni* flies did not copulate at all (Fig. 1). This suggests that *D. willistoni* is a different species from the DMH and DMP flies, as per the biological species concept, and that the amount of time populations are separated may be a practical indicator of speciation. (Biology 2 Laboratory Manual, Swarthmore College, 2017).

In terms of singing, the DMH and DMP males immediately courted the DMP females, while the *D. willistoni* males did not (Fig. 2). This suggests that singing likely evolved into a prezygotic barrier between the species, which agrees with a previous study that found that *D. willistoni* males rarely display pre-copulatory behaviors in the presence of *D. melanogaster* females (Wilson & Tompkins, 1997).

Despite their few attempts at courtship, *D. willistoni* flies did not attempt to copulate due to behavioral isolation, which necessitates a species-specific pre-copulatory behavior and morphological display to prompt mating (Ma et al. 2010). The different morphologies of each population likely influenced mate choice and served as indicators of reproductive barriers. DMP and DMH flies' similar black tail bands, coloration, and wing reflectivity reflect their short time apart, and likely contributed to their short latency to singing (Fig. 2). Their short latency to copulation resulted from their shared wing shaking behavior, the resulting vibrations, and their wing reflectivity (Fig. 1; Ma et al. 2010; Iliadi et al. 2009). *D. willistoni* flies' long latency to singing was likely due to their light color and small size (Fig. 2). Some males eventually sang to DMP females because of their few shared morphological traits, but the lack of familiar visual and auditory pre-copulatory behaviors like wing reflectivity and vibrations prevented copulation (Fig. 2; Wilson & Tompkins, 1997; Ma et al. 2010; Iliadi et al. 2009). Alternatively, copulation may not have occurred because females, as the

resource-limited sex, are more discriminating of potential mates (Wu et al. 1995). By delaying copulation with an unfamiliar male she is more likely to encounter a same-species male and can invest her energy and resources into producing young that continue her line (Biology 2 Laboratory Manual, Swarthmore College, 2017).

In the future, it would be beneficial to repeat this experiment with male DMP and female DMP, DMH, and *D. willistoni* fruit flies as previous studies have shown that *D. melanogaster* males actively woo *D. willistoni* females and occasionally copulate with them (Wilson & Tompkins, 1997). It would also be beneficial to determine the extent to which morphological differences affect mate choice when compared to behavioral differences.

Figures

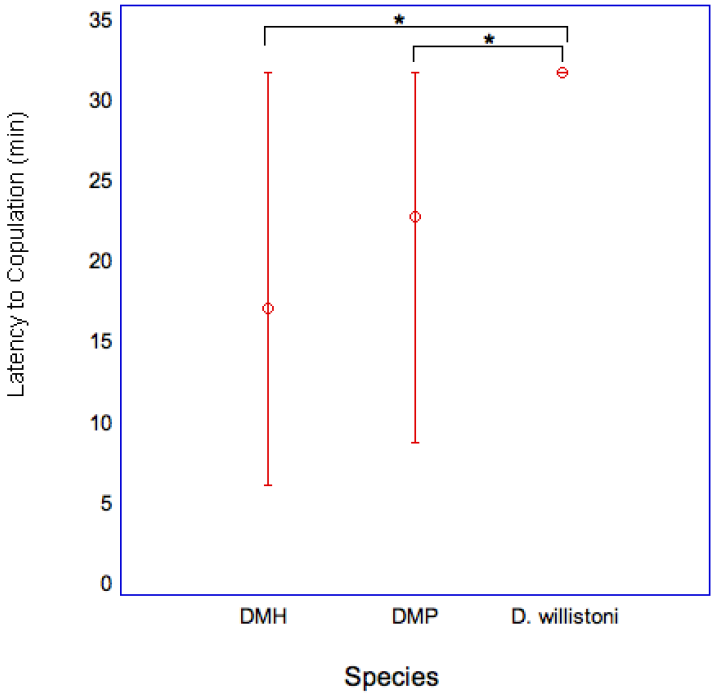


Figure 1. Median latency to singing in *D. willistoni* and *Drosophila melanogaster* from Hawaii (DMH) and Peru (DMP) fruitfly populations over a period of 30 minutes. Flies that did not copulate within the time allotted were assigned a latency of 30 minutes by default. All females were DMP fruitflies. Latency to mating behaviors and copulation were recorded every minute via scan sampling. Error bars indicate the interquartile range around the median. Kruskal-Wallis test results indicate that the differences between the three groups were statistically significant. Wilcoxin rank-sum test results with a Bonferroni correction yielding 0.016 indicate that the differences between the latencies of the DMH and DMP populations were not significant. The differences between the *D. willistoni* (n=30) and the DMP (n=38) were significant (P<0.0001). The differences between the *D. willistoni* (n=30) and DMH (n=37) were also significant (P<0.0001). Asterisks indicate significance.

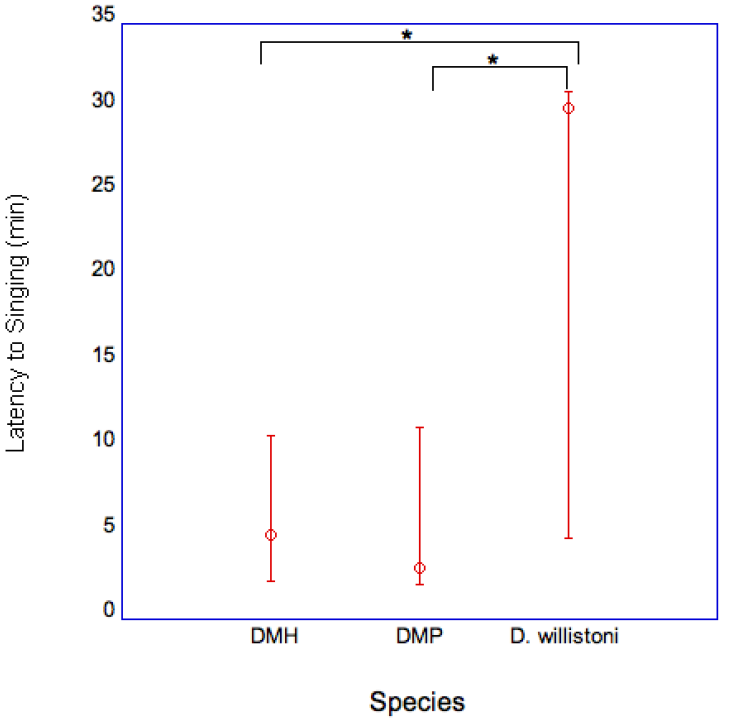


Figure 2. Median latency to singing in *D. willistoni* and *Drosophila melanogaster* from Hawaii (DMH) and Peru (DMP) fruitfly populations over a period of 30 minutes. All females were DMP fruitflies. Latency to mating behaviors and copulation were recorded every minute via scan sampling. Error bars indicate the interquartile range around the median. Kruskal-Wallis test results indicate that the differences between the three groups were statistically significant. Wilcoxin rank-sum test results with a Bonferroni correction yielding 0.016 indicate that the differences between the latencies of the DMH and DMP populations were not significant. The differences between the *D. willistoni* (n=30) and the DMP (n=28) were significant (P<0.0001). The differences between the *D. willistoni* (n=30) and DMH (n=32) were also significant (P=0.0008). Asterisks indicate significance.

Acknowledgements

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Grading Rubric for lab report final draft (25 points total)

Name of student:

_____Date:_____

Background and introduction – starts off broad and gets more specific leading into the hypotheses of interest. Adequate use of references to determine the gap of knowledge that is of interest to investigate.
Hypotheses – Clearly stated hypotheses and/or aims of the project
Materials and methods – contains all necessary details to carry out the experiment and clearly states the procedure to be used. Materials needed are included while describing the procedure.
Results – results are stated concisely and specifically, they are not interpreted, statistics are internally cited along with corresponding figures.
References – 3 or more references cited internally and formatted correctly. Reference list is provided at the end of the report.
Discussion – the results are interpreted, support of hypotheses is addressed, and speculation of results is included with the inclusion of other primary literature with internal citations. The end of the discussion becomes broad with the significance of the study being addressed.
Title – Title is creative, informative, and concise.
Formatting – 1 inch margins, 12 pt Times New Roman font, doubled line spacing, within the 4 page limit not including title, abstract, figures and references
Writing mechanics – writing is well-organized, formal, concise, and informative.

Figures – axes are labeled with units, figure caption is included, statistics are reported within the figure caption.