A Course-Based Research Approach to Human Physiology

A COURSE-BASED RESEARCH APPROACH TO HUMAN PHYSIOLOGY

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INTRODUCTION TO THE HUMAN PHYSIOLOGY LABORATORY

Introduction to Biol 256L

Karri Haen Whitmer

"Without data, you're just another person with an opinion." W. Edwards Deming

The Biology 256 Fundamentals of Human Physiology Laboratory course complements the Biology 256 lecture course and was designed to provide students with hands-on access to modern techniques in human physiological analyses using the course-based research pedagogical approach. In this course, students will learn how to perform literature searches; generate research questions and hypotheses; design experiments; collect, analyze, visualize and interpret data; and present scientific findings to others. Students gain scientific process skills by conducting experiments and/or clinical investigations each week. Around midterm, students write a series of short research proposals. The best proposal is orally presented to the class and peer-reviewed in preparation for a final, original experiment. During the last week of class, findings from the student research projects are presented to the class.

The Biol 256L curriculum offers a high-impact human physiology experience that fosters the critical thinking skills required to be a successful citizen in a modern world filled with misinformation. This goal is achieved by:

- Creating a learning environment that relies on collaborative work and emphasizes communication among staff and peers.
- Placing emphasis on collaborative assignments where students participate in experiments as experimenters and subjects.
- Focusing on course-based undergraduate research (CURE) where the literature may not be conclusive on physiological outcomes of experiments.

Learning Outcomes:

1. Develop the skills necessary to examine and interpret issues related to human physiology from an evidence-based perspective.
2. Synthesize ideas to make connections between the knowledge of anatomy, physiology and real-world problems involving human health and medicine.

Learning Goals:

1. Learn how to use common tools and procedures of a physiology laboratory, including how to use data collection hardware and analysis software.
2. Understand how to make accurate measurements of physiological phenomena, including determining sources of error.
3. Use knowledge of physiology concepts from lecture and the scientific method to propose, hypothesize about, and design experiments to test physiological phenomena.
4. Use statistics to analyze data.
5. Apply knowledge of graphs and charts to visually represent data.
6. Write and make presentations about experimental conclusions using appropriate physiological terminology.

Course Modules:

Course modules are delivered online in Canvas. Each Canvas module contains a pre-lab quiz and lab report.

Module 1: Introduction to experimental methods in human physiology research. Homework assignment: obtaining credible information from literature searches.

Module 2: Introduction to iWorx & LabScribe. Homework assignment: statistical analysis of human body temperature.

Module 3: Properties of blood. Homework assignment: data analysis & visualization.

Module 4: Body temperature homeostasis.

Module 5: Clinical techniques: performing the neurological assessment.

Module 6: Factors affecting reflex times of the Achilles and patellar stretch reflexes.

Module 7: Human nerve conduction: the nerve conduction velocity test and variables affecting conduction.

Module 8: Auditory and visual pathways and reaction times. Homework: group research proposal 1.

Module 9: Electromyography (EMG) of voluntary muscle movement. Factors affecting lever strength. Homework: group research proposal 2.

Module 10: Reading the electrocardiogram (ECG) and correlation with heart sounds. Homework: group research proposal 3.

Module 11: Breathing and gravity: factors affecting lung volumes.

Module 12: Modern uses of electrooculography (EOG) and eye tracking technologies. Homework: develop oral proposal presentation.

During the last three weeks of the course, students present final research proposals for peer review, conduct their original experiments, and present the final experimental results.

ACKNOWLEDGEMENTS

Karri Haen Whitmer

With rising education expenses it is ever more critical for university faculty to strive to reduce costs for our students. This project was developed with a love for learning and an understanding of our students' current and future needs.

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SAFETY AND COMPLIANCE IN THE HUMAN PHYSIOLOGY LABORATORY

Safety and Compliance in Biol 256L

Karri Haen Whitmer

This laboratory course requires that students fully participate in human physiology experiments or assessments each week. Because of this, several weeks require students to pay specific attention to dress code and other instructions that could affect the performance or maintenance of the equipment in the laboratory.

1. **Do not** take apart or unplug any physiology equipment. Do not disconnect sensors from iWorx hardware boxes without assistance from your instructor.
2. **Do not** disconnect any sensors from a subject's body by tugging on the wires. Instead, carefully unsnap the sensors from the electrode patch.
3. Adhesive from electrode patches is to be placed in the specified waste bins, only.
4. **Phone use in lab is strictly prohibited** except when a specific application is requested for an experiment.
5. **Do not** use lab computers for accessing unauthorized web content (any web content not required for the class).
6. Before lab, **do not** apply lotion or makeup on bodily areas where electrodes or other equipment will be placed (weeks 4,6,7,9,10,12).
7. Weekly dress code requirements:

    - **WEEK 3:** safety glasses and disposable gloves required
    - **WEEK 4:** short sleeves required, no artificial nails or nail polish
    - **WEEK 5:** examination gloves required
    - **WEEK 6:** shorts required (worn or changed into before lab)
    - **WEEK 7:** short sleeves required

- **WEEK 9:** short sleeves required
- **WEEK 11:** no lip balm, gloss or lipstick
- **WEEK 12:** no makeup on temples

SAFETY REGULATIONS FOR THE PHYSIOLOGY LABORATORY

1. Note the locations of lab safety equipment: emergency shower and eyewash station, fire extinguishers, and first aid kit.
2. Do not eat, drink, handle contact lenses, store food or beverages, or apply cosmetics in the laboratory unless you are instructed to. Please keep personal water bottles inside your bag.
3. Students who are pregnant or who have any other medical conditions (e.g. pacemaker or other implanted device) that might necessitate special precautions during lab exercises must inform the instructor.
4. Use safety glasses in experiments involving the use of hazardous liquids.
5. Wear disposable gloves when handling blood or contacting body fluids.
6. Keep all liquids away from the edge of the table to avoid spills. Keep all liquids away from electronic hardware and outlets. Report any spills or accidents to your instructor immediately.
7. Dispose of waste properly. Sharps (syringes, scalpel blades, etc.), glass, materials contaminated with body fluids, and animal tissues have specific disposal bins in the A&P laboratories. Do not put these in the trash.
8. Clean up and organize at the end of every lab period. Put away physiology lab equipment at your station so that it is in the same condition you found it.

For additional information pertaining to laboratory safety at ISU, please see the following publication: Laboratory Safety Manual [pdf]

Link here for general safety/emergency response information: Student Emergency and Safety Information (external link)

SCIENTIFIC METHODS AND HUMAN SUBJECTS RESEARCH

Introduction to Experimental Methods

Karri Haen Whitmer

Our understanding of the methods used to conduct good scientific research is important for progress in our scientific understanding but also impacts our daily lives. Understanding good scientific methodology allows us to not only conduct experiments, but it helps us to analyze research conducted by others. For example, it helps us to determine whether research studies reported in the news are reliable. Research knowledge also helps us to discriminate among different medical treatments when it comes to making personal health decisions.

Scientific research methods include several steps, which may differ depending upon the topic to be addressed by a study. Standard scientific methods typically include: definition of the research problem, conducting background research, formulation of hypotheses, designing and conducting experiments, analysis of results, formulation of conclusions, and communication of research results to the public.

Central to our acquisition of scientific knowledge is the concept of the experiment. Researchers do **experiments** to answer questions about the world around us. The following are examples of simple **research questions** in human physiology:

1. Does changing the respiratory rate affect heart rate?
2. Does caffeine consumption affect blood glucose levels?
3. Does body temperature affect blood oxygen levels?

In order to answer these questions, researchers begin by formulating testable **hypotheses**. A hypothesis is a *tentative* statement describing the relationship between the variables in an experiment. Research hypotheses are written as if/then statements that include dependent and independent variables.

A variable is any factor that can change, affecting the experimental results. The **dependent variable** is the variable in the experiment that is measured by the researcher. The **independent variable** is the variable that is manipulated by the researcher in order to exert an effect on the dependent variable. In the first example research question, the heart rate is the dependent variable, and the respiratory rate is the independent variable. The researcher will use an **experimental method** (for example deep breathing) to manipulate a subject's respiratory rate to measure whether any changes occur in the heart rate.

Dependent variable: the variable that is measured as the output of an experiment (the result)

Independent variable: a variable that is manipulated by the researcher

Writing Hypotheses

A hypothesis is a "tentative statement that proposes a possible explanation to some phenomenon or event."[1] Hypotheses written for the purpose of conducting experiments must be testable. **Formalized hypotheses** use an if/then format that helps to assure that all important aspects of the hypothesis are intact, including the independent and dependent variables. Additionally, a good **research hypothesis** has three parts: an explanation of a phenomenon to be tested, a method, and a prediction. A research hypothesis must be written before an experiment is conducted.

1. http://www.accessexcellence.org/LC/TL/filson/writhypo.html

A research hypothesis includes a potential explanation for a phenomenon, a method, and a prediction.

Imagine students working on a physiology project involving muscle contraction and temperature. The students observe that cold hands do not function as well at performing certain tasks requiring manual dexterity than do warm hands. The students decide to test grip strength under different temperature conditions using a handgrip dynamometer, which measures the strength of contraction of hand and forearm muscles.

The following are examples of bad and good **research hypotheses** for this experiment:

1. *My grip strength will be stronger with warm hands than with cold hands.*

This example is not a research hypothesis because it only includes a prediction. A prediction by itself is never a formalized hypothesis.

2. *If I test grip strength with a handgrip dynamometer, then my grip strength will be stronger with warm hands than with cold hands.*

This example is not a research hypothesis because it only includes a method (a test) and a prediction. It does not include any explanation of the phenomenon to be tested.

3. *If low temperatures suppress muscle contraction, and I test grip strength at different temperatures with a handgrip dynamometer, then my grip strength will be stronger with warm hands than with cold hands.*

This is an example of a correct research hypothesis. Note the three parts: "if low temperatures suppress muscle contraction" (a possible explanation of the phenomenon to be tested), "and I test grip strength at different temperatures with a

handgrip dynamometer" (the method used for the test), and "then my grip strength will be stronger with warm hands than with cold hands" (the prediction).

This writing sample is also an example of a formalized hypothesis due to the use of the if/then format. In this hypothesis, the independent variable is muscle temperature, and the dependent variable is muscle contraction strength.

Exercise: Practice writing a research hypothesis

Background: The ad for a creatine supplement claims ingesting 10g of creatine once a day for four weeks results in measurable increases in muscle mass. A student decided to test the claim in 10 subjects by measuring the circumference of the upper arm, around the belly of the biceps muscles, before and after treatment. The subjects were not allowed to take part in weight or resistance training during the testing period.

Write a hypothesis as an if/then statement for this experiment:

What is the dependent variable?

What is the independent variable?

Designing Experiments involving Humans

Well-designed experiments must minimize the effects of extraneous environmental and physiological factors, in order to make sure changes recorded in the dependent variable are actually the result of manipulating the independent variable. **Experimental controls** establish a baseline for the experiment. When conducting **human subject experiments** in physiology, the **control** might consist of a separate

group of people, the **control group**, who are not exposed to any manipulation of the independent variable, or it might be the same group of subjects tested before (and then after) altering the independent variable.

Experimental studies may be *in vitro*, conducted in highly controlled laboratory conditions (example: in a test tube), or *in vivo*, conducted in a live organism. Controlled laboratory experiments (also called "bench research," molecular, or cellular research) allow for a great amount of control over the variables that could affect experimental outcomes because all the components in the experimental system can typically be easily accounted for and measured. In **human subject research**, studies that use human participants to answer a research question, there is typically much less control over experimental variables due to the natural anatomical, physiological, and environmental variation innate to human populations. These are called **external variables** and can profoundly affect the outcome of an experiment. For example, two subjects may metabolize a compound differently due to differences in enzymes or two subjects that may react to cardiovascular stress differently due to their sex, age, or fitness level. To account for these external, or uncontrolled, variables in human subjects, experiments often use a within-subjects design (below) where the dependent variable is measured in the same subjects before and after manipulating the independent variable.

In human subjects research, there are two main types of experimental designs: within-subjects design and between-subjects design. In a **within-subjects design**, the subjects of the study participate under each study condition, including in the control group. In the most simplistic design, the subjects participate in baseline measurements for the control (no treatment) and then participate under experimental conditions. Because the subjects in this kind of study serve as their own control group, variation in the results due to many external variables can be reduced.

An example of a simple within-subjects design can be found in many pharmaceutical studies where a group of participants is given a placebo drug for a defined amount of time, and then the same group is given an experimental drug. Differences in physiological measurements after treatment with the experimental drug are inferred as effects of drug administration.

One disadvantage of this research design is the problem of **carryover effects**, where the first test adversely influences the other. Two examples of this, with opposite effects, are fatigue and practice. In a complicated experiment, with multiple treatment conditions, the participants may be tired and thoroughly fed up of researchers prying and asking questions and pressuring them into taking tests. This could decrease their performance on the last study.[2]

Alternatively, the practice effect might mean that they are more confident and accomplished after the first condition, simply because the experience has made them more confident about taking tests. As a result, for many experiments, a counterbalance design, where the order of treatments is varied, is preferred, but this is not always possible.

Another type of experimental design is the **between-subjects design**. In the between-subjects design, there are separate participants for the control and treatment groups, which avoids carryover effects. However, the between-subjects design may make it impossible to maintain homogeneity across the groups: age, gender, and social class are just some of the obvious factors that could result in differences between control and treatment groups, skewing the data.

Within-subjects design: the subjects in the study participate in the control and treatment conditions

Between-subjects design: different groups of subjects participate in the control and treatment conditions

2. Martyn Shuttleworth (May 16, 2009). *Within Subject Design*. Retrieved Jul 30, 2019 from Explorable.com: https://explorable.com/within-subject-design Creative Commons-License Attribution 4.0 International (CC BY 4.0).

Experimental Error

No matter how careful we are in creating an experimental design, no experiment can be perfect. We must assume there is some margin of error in the collected data. There are three general types of errors that can impact the outcome of an experiment:

1. **Human error:** human errors are simple mistakes made by an experimenter. For example, the experimenter didn't appropriately attach a sensor or read a patient's blood pressure wrong.
2. **Systematic error:** systematic error is often due to poor experimental design or instrument error (poorly calibrated instrument, etc.). Systematic errors include sampling bias, selection bias, and measurement bias.

    - **Sampling bias:** the participants in the study are not representative of the population at large; thus, the results cannot be generalized outside of the study population. For example, data from a study conducted on only 80-year-old men may not be generalized to everyone else in the human population.
    - **Selection bias:** the assignment of subjects to control and treatment groups was not random, resulting in experimental results highly impacted by external variables. For example, a control group that included only females and a treatment group that contained only males.
    - **Measurement bias:** the experimenters rate subjects differently due to their own expectations of experimental outcomes.

3. **Random error:** by-chance variations in measurements that cannot be controlled. Random errors can be reduced by repeated measurements.

The box below lists some sources of error that are possible in all human subject experiments.

Common factors adversely affecting the outcome of human subject experiments:

- Subjects in the study are not representative of the human population at large: e.g., small sample size is too small to fully account for variation in the population
- Interference due to external variables
- Problems with the reliability or accuracy of instruments: e.g., equipment does not have the precision to detect changes in the dependent variable
- Human error: the researcher makes an erroneous measurement or other error

CONDUCTING BACKGROUND RESEARCH IN THE SCIENCES

Literature Searches

Karri Haen Whitmer

Before addressing a research problem with an experiment, it's important to conduct background research in order to learn what is already known about the problem. It's a good idea to start any research project by making use of the resources at your institution's library. The Iowa State University Library has several resource guides available that are specific for an area of study. These can make it much easier to search for the appropriate information for a particular type of research question. Here is the resource guide for Kinesiology at ISU: Kinesiology Research Guide at Iowa State University (external link)

Resource Types

There are many types of resources that may be valuable for a literature search. **Primary literature** includes original written works such as research published in scholarly journals. Primary literature is the ideal resource for academic work; however, the terminology used may be difficult to understand for beginners in a field of study. **Secondary sources** include books or review articles that summarize primary research findings. A good example of a secondary resource is a textbook. Today, various **internet resources** are popular for conducting research. For information on how to wisely use internet resources, please see the section on evaluating internet resources at the end of this chapter.

How to Read a Scientific Article

Reading a scientific article is a complex task.[1] The worst way to approach this task is to treat it like the reading of a textbook—reading from title to literature cited, digesting every word along the way without any reflection or criticism. Rather, you should begin by skimming the article to identify its structure and features. As you read, look for the author's main points. Generate questions before, during, and after reading. Draw inferences based on your own experiences and knowledge. And to really improve understanding and recall, take notes as you read. This handout discusses each of these strategies in more detail.

Skim the article and identify its structure

Most journals use a conventional IMRD structure: An abstract followed by Introduction, Methods, Results, and Discussion. Each of these sections normally contains easily recognized conventional features, and if you read with an anticipation of these features, you will read an article more quickly and comprehend more.

Features of Abstracts

Abstracts usually contain four kinds of information:

- purpose or rationale of study (why they did it)
- methodology (how they did it)
- results (what they found)
- conclusion (what it means)

Most scientists read the abstract first. Others—especially experts in the field—skip right from the title to the visuals because the visuals, in many cases, tell the reader what kinds of experiments were done and what results were obtained. You should probably begin reading a paper by reading the abstract carefully and noting the four kinds of information outlined above. Then move first to the visuals and then to the rest of the paper.

Features of Introductions

Introductions serve two purposes: creating readers' interest in the subject and providing them with enough information to understand the article. Generally, introductions accomplish this by leading readers from broad information (what is known about the topic) to more specific information (what is not known) to a focal point (what question the authors asked and answered). Thus, authors describe previous work that led to current understanding of the topic (the broad) and then situate their work (the specific) within the field.

Features of Methods

The Methods section tells the reader what experiments were done to answer the question stated in the Introduction. Methods are often difficult to read, especially for graduate students, because of technical language and a level of detail sufficient for another trained scientist to repeat the experiments. However, you can more fully understand the design of the experiments and evaluate their validity by reading the Methods section carefully.

Features of Results and Discussion

The Results section contains results—statements of what was found, and reference to the data shown in visuals (figures and tables). Normally, authors do not include information that would need to be referenced, such as comparison to others' results. Instead, that material is placed in the Discussion—placing the work in context of the broader field. The Discussion also functions to provide a clear answer to the question posed in the Introduction and to explain how the results support that conclusion.

Distinguish the Main Points

Because articles contain so much information, it may be difficult to distinguish the main points of an article from the subordinate points. Fortunately, there are many indicators of the author's main points:

Document level

- Title
- Abstract
- Keywords
- Visuals (especially figure and table titles)
- First sentence or the last 1-2 sentences of the Introduction

Paragraph level: words or phrases to look for

- surprising
- unexpected
- in contrast with previous work
- has seldom been addressed
- we hypothesize that
- we propose
- we introduce
- we develop
- the data suggest

Generate questions and be aware of your understanding

Reading is an active task. Before and during your reading, ask yourself these questions:

- Who are these authors? What journal is this? Might I question the credibility of the work?
- Have I taken the time to understand all the terminology?
- Have I gone back to read an article or review that would help me understand

this work better?
- Am I spending too much time reading the less important parts of this article?
- Is there someone I can talk to about confusing parts of this article?

After reading, ask yourself these questions:

- What specific problem does this research address? Why is it important?
- Is the method used a good one? The best one?
- What are the specific findings? Am I able to summarize them in one or two sentences?
- Are the findings supported by persuasive evidence?
- Is there an alternative interpretation of the data that the author did not address?
- How are the findings unique/new/unusual or supportive of other work in the field?
- How do these results relate to the work I'm interested in? To other work I've read about?
- What are some of the specific applications of the ideas presented here? What are some further experiments that would answer remaining questions?

Draw inferences

Not everything that you learn from an article is stated explicitly. As you read, rely on your prior knowledge and world experience, as well as the background provided in the article, to draw inferences from the material. Research has shown that readers who actively draw inferences are better able to understand and recall information.

Template for Taking Notes on Research Articles: Easy access for later use

Whenever you read an article, pertinent book chapter, or research on the web, use the following format (or something similar) to make an electronic record of your notes for later easy access. Put quotation marks around any exact wording you write down so that you can avoid accidental plagiarism when you later cite the article.

Complete citation. Author(s), Date of publication, Title (book or article), Journal, Volume #, Issue #, pages:

If web access: url; date accessed

Key Words:

General subject:

Specific subject:

Hypothesis:

Methodology:

Result(s):

Summary of key points:

Context (how this article relates to other work in the field; how it ties in with key issues and findings by others, including yourself):

Significance (to the field; in relation to your own work):

Important Figures and/or Tables (brief description; page number):

Cited References to follow up on (cite those obviously related to your topic AND any papers frequently cited by others because those works may well prove to be essential as you develop your own work):

How to Spot Fake News

Figure 2. How to spot fake news by IFLA. There are several questions you should ask yourself when consulting a resource.

Wikipedia

Wikipedia is broadly misunderstood by faculty and students alike.[2] While Wikipedia must be approached with caution, especially with articles that are covering contentious subjects or evolving events, it is often the best source to get a quick, consensus viewpoint on a subject. Because the Wikipedia community has strict rules about sourcing facts to reliable sources, and because authors are asked to adopt a neutral point of view, its articles are often a good introduction to a subject on the web. However, be advised that *anyone* can edit Wikipedia, and those who write or add to articles may not be experts. Sometimes the claims in Wikipedia articles are blatantly erroneous.

Despite this, the focus on sourcing claims in Wikipedia has a beneficial effect. If you can find a claim expressed in a Wikipedia article, you can follow the footnote on the claim to a reliable source, which may be a primary resource. In this way, scholars can benefit from using Wikipedia to quickly find authoritative sources for claims, and use these primary resources as a starting point for investigating a question.

Evaluating Internet Resources: the CRAAP Test

When you search for information, you're going to find plenty... but is it accurate and reliable (Fig. 1)? [3] You will have to determine this for yourself, and the CRAAP Test can help. The CRAAP Test is a list of questions to help determine if the information you find is good quality. Your information source may not meet every criterion on this list; different criteria will be more or less important depending on your situation or need. So why guess? Is your source giving you truly credible and useful information or just fake news?

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2. Adapted from *Web literacy for student fact checkers*, by Michael A. Caulfield. CC by 4.0 International license
3. Adapted from https://uri.libguides.com/start/craap CC by 4.0 International license.

Currency: The timeliness of the information.

- When was the information published or posted?
- Has the information been revised or updated?
- Is the information current or too out-of-date for my topic?
- Are all the links functional or are there dead links?

Relevance: The importance of the information for your needs.

- Does the information relate to my topic or answer my question?
- Who is the intended audience?
- Is the information at an appropriate level (i.e. not too simple or advanced) for my needs?
- Did I look at a variety of sources before deciding to use this one?
- Would I be comfortable using this source for my college research paper?

Authority: The source of the information.

- Who is the author/publisher/source/sponsor?
- Are the author's credentials or organizational affiliations given?
- What are the author's credentials or organizational affiliations?
- What are the author's qualifications to write on the topic?
- Is there contact information, such as a publisher or e-mail address?
- Does the URL reveal anything about the author or source? Examples: .com .edu .gov .org .net

Accuracy: The reliability, truthfulness, and correctness of the information.

- Where does the information come from?
- Is the information supported by evidence?
- Has the information been reviewed by anyone else?
- Can I verify any of the information in another source or from personal knowledge?
- Does the language or tone seem biased? Or is it free of emotion?

- Are there spelling, grammar, typographical, or other errors?

Purpose: The reason the information exists.

- What is the purpose of the information? to inform? teach? sell? entertain? persuade?
- Do the authors/sponsors make their intentions or purpose clear?
- Is the information fact? opinion? propaganda?
- Does the point of view appear objective and impartial?
- Are there political, ideological, cultural, religious, institutional, or personal biases?

VITAL SIGNS AND BMI

Week 1 Laboratory Background and Methods

Karri Haen Whitmer

Vital signs are measurements of the body's most basic functions. The four main vital signs routinely monitored by medical professionals include the following:

- **Body temperature** in degrees Celsius (°C) or degrees Fahrenheit (°F)
- **Pulse rate** in beats per minute (bpm)
- **Respiration rate** or rate of breathing in breaths per minute (bpm)
- **Blood pressure**

Comparing patients' vital sign measurements to the normal range of values is useful for detecting medical problems. In this lab, we will also assess your Body Mass Index (BMI) or body composition. Keeping track of the BMI is an excellent way to achieve numerous health benefits, such as helping to reduce the risk of cardiovascular disease.

Lab 1 Learning Objectives

In this lab, you will learn the basic methods for determining a patient's vital signs.

- Body temperature measurement and conversion
- Determination of pulse rate rate per minute
- Measurement of breathing rate per minute
- Taking and analyzing blood pressure readings
- Measurement and analysis of body weight and body fat composition

What is normal body temperature?

The body temperature of a person fluctuates depending on environmental temperature, activity, food and fluid consumption, time of day, and, in women, the stage of the menstrual cycle. Although the average human body temperature is usually stated as 37°C (98.6°F), a systematic review of the literature has found normal oral body temperature can range from 91.76-100.76°F in healthy adults (Sund-Levander et al., 2002). With this in mind, it can be important to compare body temperature readings to baseline values for individuals.

Body temperature readings may reflect the core body temperature (temperature of the internal organs) or the temperature of the body's shell (body surfaces which change temperature due to environmental exposure). Typically, the core body temperature is slightly higher than the shell temperature.

A person's body temperature can be measured in several ways:

- **Orally.** Temperature can be taken by mouth using either the classic glass thermometer or modern digital thermometers that use an electronic probe. The average normal oral temperature is 98.6°F (37°C).
- **Rectally.** Temperatures taken rectally (using a glass or digital thermometer) measure core body temperature and are typically0.5°F (0.3°C) to 1°F (0.6°C) higher than the oral temperature.
- **Axillary.** Temperatures can be taken under the arm using a glass or digital thermometer. An axillary temperature is usually 0.5°F (0.3°C) to 1°F (0.6°C) lower than the oral temperature.
- **By ear.** A special thermometer can quickly measure the temperature of the ear drum, which reflects the body's core temperature (the temperature of the internal organs). Tympanic temperature is 1°F (0.6°C) higher than the oral temperature.
- **By skin.** A special thermometer can quickly measure the temperature of the skin on the forehead. A forehead (temporal) scanner temperature is usually 0.5°F (0.3°C) to 1°F (0.6°C) lower than the oral temperature.

Body temperature may be abnormal due to fever (high temperature) or hypothermia (low temperature). **A**ccording to the American Academy of Family

Physicians, fever is indicated when body temperature rises one degree or more over the **normal temperature of 98.6°F** (or one degree over the individual's baseline temperature). Hypothermia is defined as a reduction in body temperature below 95°F.

In the laboratory, you may need to convert between Celsius and Fahrenheit temperature readings.

Convert Celsius to Fahrenheit: $°F = (°C \times 9/5) + 32$ or $°F = (°C \times 1.8) + 32$

Convert Fahrenheit to Celsius: $°C = (°F - 32) / (9/5)$ or $°C = (°F - 32) / 1.8$

The Components of the Body's Thermoregulatory System

The body's thermoregulatory efforts are coordinated by a number of different centers in the brain, the most important of which is the *hypothalamus,* a region located in the base of the brain just above the pituitary gland. Input to these brain centers comes from the thermoreceptors located within the brain itself. There, receptors, known as *central thermoreceptors,* are important because they monitor the temperature deep within the body (known as **core temperature**). Other thermoreceptors, called *peripheral thermoreceptors,* are located in the skin and detect more variable temperatures found at the body's surface.

Output from brain thermoregulatory centers is transmitted by neurons to various effectors that vary the rate of heat production or loss in order the stabilize body temperature. The effectors include *sweat glands*, which control evaporative heat loss by increasing or decreasing sweat secretion; *blood vessels in the skin,* which control conductive and radiative heat loss by increasing or decreasing the blood flow to the skin's surface; and *skeletal muscles*, which control heat production through *shivering*– involuntary contractions that generate heat as a metabolic by-product.

When the core temperature changes, brain thermoregulatory centers attempt to compensate by sending appropriate commands to these effectors.

How body temperature is maintained within the normal range. The body temperature normally has a set point of 37°C (98.6°F). During exposure to ambient (environmental) temperatures between 25-30°C (called the ***thermoneutral zone***), altering the flow of blood through the skin is enough to stabilize core body temperature. Blood vessels near the body's surface constrict to conserve body heat when the temperature is too cool – this process of decreasing blood vessel diameter to restrict blood flow is called **vasoconstriction**. The same cutaneous vessels dilate to radiate heat from the skin when the body is too warm. Increasing the blood vessel diameter is called **vasodilation**. When cutaneous vessels vasodilate, they radiate excess heat from the skin to the external surroundings.

When body temperature begins to diverge farther away from the set point, two antagonistic mechanisms can bring it back to a normal value: shivering and sweating. Shivering is induced when the body temperature falls too low, and subsides through negative feedback when the temperature returns to normal. Sweating occurs when the body temperature is too high, and it diminishes as the core body temperature falls.

What is the pulse rate?

The pulse rate is a measurement of the heart rate, or the number of times the heart beats per minute. As the ventricles of the heart contract and pump blood through the arteries, the arteries expand and contract with the flow of the blood. Taking a pulse not only measures the heart rate, but also can indicate the following:

- Heart rhythm
- Strength of the pulse (strength of heart contraction)

The normal resting pulse for healthy adults, ranges from 60 to 100 beats per minute. The pulse rate may fluctuate with exercise, illness, injury, and emotions. Females ages 12 and older, in general, tend to have faster heart rates than do males. Athletes, such

as runners, who do a lot of cardiovascular conditioning, may have heart rates near 40 beats per minute.

As the ventricles of the heart contract and force blood through the arteries, you can feel the beats by pressing on the arteries, which are located close to the surface of the skin at certain points of the body. The pulse can be found on either side of the neck, at the carotid arteries, at the cubital fossa in the antecubital area on the brachial artery, or at radial artery at the wrist. For most people, it is easiest to take the pulse at the radial artery. If you use the carotid arteries in the neck, be sure not to press too hard, and never press on both arteries on the sides of the lower neck at the same time to prevent blocking blood flow to the brain.

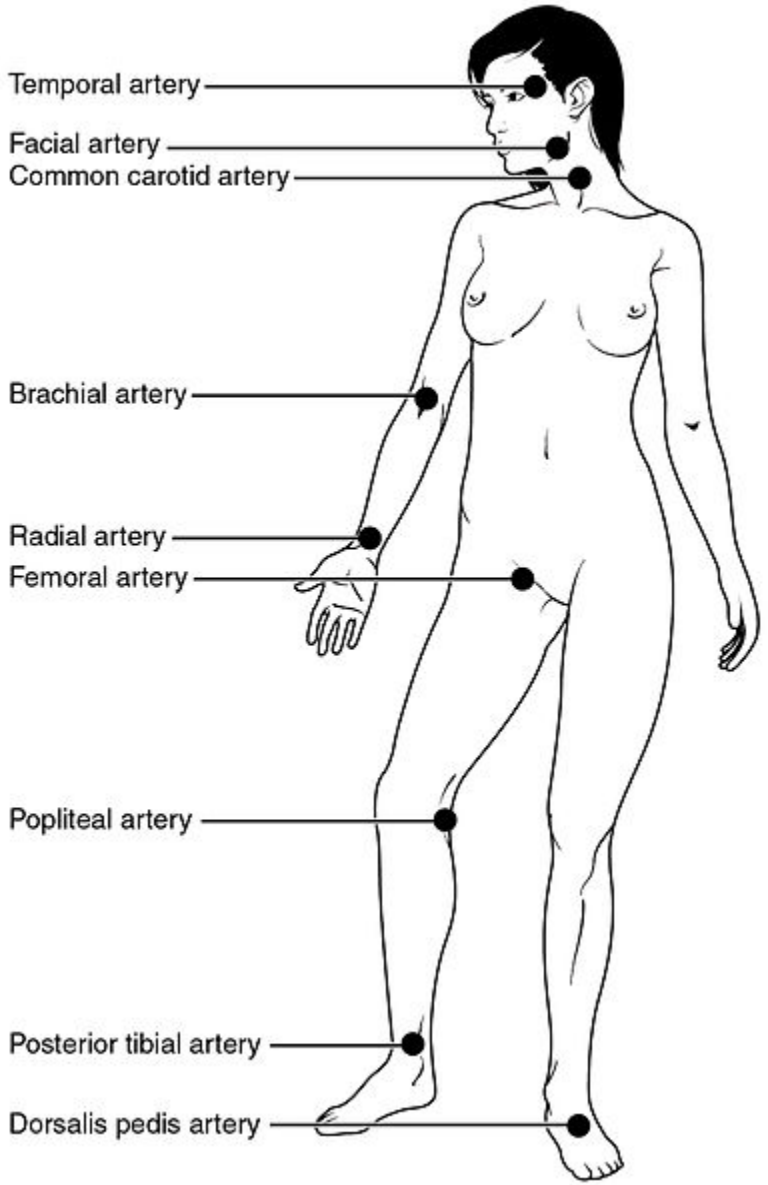


Figure 1. Common sites for taking the pulse on the human body. Illustration from OpenStax Anatomy & Physiology, licensed CC BY 4.0.

Your pulse indicates the number of contractions made by the left ventricle of your heart each minute. Your heart controls your heart rate intrinsically, with each contraction. No nervous stimulation is required in order for the heart to contract.

When taking your pulse at the wrist: place two fingers over the radial artery (Figure 1) on the lateral side of your wrist. When you find the pulse, count the number of beats for 60 seconds (or count for 20 seconds and multiply by 3).

Although the heart muscle is autorhythmic, it responds to the controls of the autonomic nervous system (ANS). The sympathetic nervous system may increase the heart rate and pulse under stressful situations such as emotional trauma, physical exertion or injury with blood loss (hypovolemic shock) and/or infection. A resting heart rate over 100 bpm is considered **tachycardia**, which, if prolonged, may be a sign of coronary disease. In the case of blood loss, an elevated heart rate is a compensatory response to hemorrhage. Decreased arterial pressure due to low blood volume initiates the **baroreceptor reflex**, which increases sympathetic stimulation of the heart, increasing heart rate, cardiac output and arterial pressure. On the other hand, the parasympathetic nervous system *always modifies the heart rate* by decreasing it via the Vagus nerve.

What is the respiration rate?

The **respiration rate** is the number of breaths a person takes per minute. Normal respiration rates for an adult person at rest range from 12 to 18 breaths per minute. The rate is usually measured when a person is at rest and simply involves counting the number of breaths for one minute by observing how many times the chest rises. **Respiration rates may increase with fever, illness, trauma, and other medical conditions.** In addition, note that lung sounds observed with a stethoscope should be fluid without any clicking, bubbling, rattling or wheezing, as these also indicate potential disease.

Respiration allows your lungs to acquire oxygen and eliminate carbon dioxide. Oxygen is used in cellular respiration as the final electron acceptor when mitochondria make the ATP that is used as fuel for cellular work. Carbon dioxide is made as you break down the body's fuel molecules to make ATP. When you are doing more cellular work such as exercising, your respiratory rate increases because

you require more ATP and produce more carbon dioxide. Your medulla oblongata sets the respiratory rhythm by alternately stimulating inspiration (inhaling) and expiration (exhaling). Respiratory rhythm is modified by the pons to correct for activities such as exercising or talking.

What is blood pressure?

Your **Systemic Blood Pressure** measurement indicates the pressure in your peripheral arteries when the left ventricle of the heart is pumping blood to systemic arteries, called the **systolic pressure**, as well as the pressure in the systemic arteries when the ventricles are relaxing and filling with blood, called the **diastolic pressure**. Blood pressure is reported as systolic pressure over diastolic pressure. The units (mm Hg) cancel each other out and are not reported.

Pulse pressure is the difference between the systolic and the diastolic pressures. For example, if your Blood Pressure is 120/80, the pulse pressure would be 120-80=40. In older adults (60+ years) a pulse pressure greater than 60 can be an indicator of cardiovascular disease, a predictor of heart attacks, and may also reflect leaky heart valves due to age-related losses in aortic elasticity. In turn, a pulse pressure less than 40 may indicate poor heart function.

Systolic and diastolic pressure should be considered alongside pulse pressure values. Higher systolic and diastolic pairs imply higher risk than lower pairs with the same pulse pressure: 160/120 indicates a higher risk than 110/70 even though the pulse pressure in each pair is 40.

The most important cause of elevated pulse pressure is the stiffness of the aorta. The stiffness may be due to high blood pressure or fatty deposits damaging the walls of the arteries, leaving them less elastic (atherosclerosis). The greater your pulse pressure, the stiffer and more damaged the vessels are thought to be.

Three important factors affect Blood Pressure: Cardiac Output, Peripheral Resistance and Blood Volume. Increasing any of these factors will increase blood pressure.

Equation 1: Arterial pressure = cardiac output X peripheral resistance

- **Cardiac Output** is the volume of blood pumped per minute; both the heart rate [beats per minute (bpm)] and the stroke volume (volume pumped per minute) affect it.
- **Peripheral Resistance** is the resistance of arteries to blood flow and is affected by three factors: vessel length, diameter, and the viscosity of the blood. Increasing the peripheral resistance through any of these factors increases blood pressure:

1. **Vessel length**: When blood vessels lengthen, peripheral resistance increases. Adipose tissue mass is an important factor, in that, miles of small blood vessels supply each pound fat. Therefore, extra adipose tissue increases peripheral resistance and blood pressure.
2. **Vessel diameter**: When blood vessel diameter *decreases*, peripheral resistance increases. Blood vessel diameter is increased via vasodilation to deliver more blood to active tissues. When vasoconstriction occurs, the blood flow is more turbulent and the blood pressure increases.
3. **Blood viscosity**: Increasing blood viscosity or "stickiness" increases peripheral resistance. When there are elevated amounts of blood cells or blood proteins, the blood becomes more viscous and is not easily pumped, causing elevated blood pressure

- **Blood Volume**, the total blood volume in the average-size adult (150-160 lbs), fluctuates but averages about 5 liters, constituting approximately 8% of the total body weight. *Blood volume may decrease below normal values due to blood loss or dehydration.* A reduced total blood volume will reduce the cardiac output (volume pumped per minute) by decreasing venous blood return to the heart. Blood volume may increase beyond normal values due to pregnancy and/or excess water and sodium retention (which may be due to a high sodium diet or other physiological factors).

The 2017 Blood Pressure Guidelines

In 2017, the American Heart Association amended previous blood pressure recommendations. The changes are summarized in the table (below). The American Heart Association currently defines a normal healthy adult blood pressure as a blood pressure with **systolic pressure** less than 120 mm Hg and a **diastolic pressure** less than 80 mm Hg. A normal blood pressure of 117/79 is read as "117 over 79."

Blood pressure is considered **elevated** if the systolic pressure is between 120-129 and the diastolic pressure is less than 80 mm Hg. **Hypertension** is when the pressures, either systolic or diastolic, are greater than these parameters. Temporary elevations in blood pressures are normal with exciting events, disturbing situations and physical exertion. Long-term hypertension, referred to as chronic hypertension over-works the heart leading to a weakening of the heart's pumping efficiency. Chronic hypertension is called a "silent killer" as damage to the heart has taken place before it is detected.

Blood Pressure Categories

American Heart Association | American Stroke Association

BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)		DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

heart.org/bplevels

Figure 2. Blood Pressure Categories according to the American Heart Association. Normal blood pressure has a systolic value less than 120 and a diastolic value less than 80. Graphic courtesy of the American Heart Association.

Hypotension occurs when blood pressure is too low. In most cases, the lower the blood pressure, the better. If an individual has a systolic pressure under 90 or diastolic pressure under 60 and is experiencing certain chronic, low blood pressure

symptoms such as dizziness, nausea, fainting or blurred vision, they may be diagnosed with hypotension. In this case, the underlying cause must be established. On the other hand, **acute hypotension** may occur with blood loss or severe dehydration and may constitute a medical emergency.

Body position and BP measurement: Blood pressure may be measured in the sitting, standing or supine (lying down) position. However, it should be noted that blood pressure results are affected by body position and arm position (at heart level or above/below heart level). For example, it is widely accepted that blood pressure is highest in the supine position. While standing, the pull of gravity causes blood to pool in the veins of the legs, somewhat reducing venous return to the heart. Reduced venous return reduces cardiac output and thus reduces the arterial pressure in a standing patient (see Equation 1). This drop in blood pressure is transient in normal individuals because the **baroreceptor reflex,** which initiates sympathetic stimulation of heart contraction, increases heart rate and blood pressure in response to low pressure values.

A good clinical example of changing body position and blood pressure is that of **orthostatic, or postural, hypotension**. In orthostatic hypotension, an individual may experience an exaggerated fall in blood pressure when moving from a supine (or sitting) to a standing position, leading to light-headedness or syncope. Orthostatic hypotension is also normally resolved by the **baroreceptor reflex**.

What is Body Mass Index (BMI)?

BMI measures the amount of fat in your body relative to your height and weight. Percentage of body fat is strongly associated with the risk of several chronic diseases, such as hypertension, dyslipidemia (a condition marked by abnormal concentrations of lipids or lipoproteins in the blood), diabetes mellitus and coronary heart disease. Your body composition can be found by a number of methods; we will utilize Bioelectrical Impedance and Body Mass Index (BMI).

Bioelectrical Impedance Analysis (BIA) is based on the principle that electric current flows at different rates through body tissues depending upon their composition. The body is composed mostly of water and ions, through which an electric current can flow. **Total body water** is contained in two compartments:

extra-cellular water (ECW, approximately 45% and **intracellular water** (ICW, approximately 55%). The body is also composted of non-conducting materials, such as body fat (adipose tissue), which provide resistance to the flow of electric current. Adipose tissue contains only about 20% water, so it impedes (slows) the flow of electric current; thus, it is considered to have high impedance. Muscle contains about 75% water and allows the electric current to pass through at a faster rate, so it has low impedance. The bioelectrical impedance of the entire body, therefore, reflects the proportion of body fat to fat-free tissue (muscle and bone).

Since, the principle of BIA is that electric current passes through the body at a different rate depending on body composition, there is a direct relationship between the concentrations of ions and the electrical *conductivity* and an indirect relationship between ion concentrations and the *resistance* of the solution. Our findings will be based on the principle that adipose tissue impedes electric current more than muscle and bone.

Body Mass Index (BMI) is a person's weight in kilograms divided by the square of body height in meters.

Table 1. BMI and Weight Status

BMI for Adults	Weight Status
Below 18.5	Underweight
18.5—24.9	Normal
25.0—29.9	Overweight
30.0 and Above	Obese

Normal percentage of body fat is dependent on age and sex. The table below shows the percentage of body fat changes continuously as one ages, and it is different depending on sex. In females, sex hormones will affect the percentage of body fat

through the lifespan. Physical activities may also affect body composition and the percentage of body fat.

Table 2. Body Fat Percentages-Normal Values for Average Individuals

Age	18-39 years	40-59 years	60-99 years
Females	21-33%	23-34%	24-36%
Males	8-20%	11-22%	13-25%

Laboratory Methods

Week 1 Laboratory Exercises

The following materials provide instructions for completing laboratory 1.

Download your lab report from Canvas to record your vital signs data.

Part 1. Body Temperature

Normal adult body temperature is about 98.6 degrees Fahrenheit (°F) or 37 degrees Celsius (°C), but body temperature often varies from 1 to 2 °F or ½ to 1 °C throughout the day.

Measuring Human Body Temperature Using a Digital Thermometer:

- Before starting, clean the thermometer with 70% Isopropyl Alcohol pad and

allow the probe to dry.

- Place a new disposable probe cover over the probe before each use.
- Press the power button.
- If the next screen only shows °F, you will need to *calculate and record* the oral temperature in °C as well.
- Place the digital thermometer probe in your mouth with the tip pressed firmly under the tongue. Close your lips around thermometer and wait until the thermometer beeps.
- Record the oral temperature in °F & °C.
- **Complete three total replicates, recording oral temperatures in both °F & °C on the lab report. Calculate and record the mean of the three trials.**
- Push the power button to clear the reading and remove the disposable probe cover. Wipe the thermometer probe with an isopropyl alcohol pad, and return it to its plastic case.

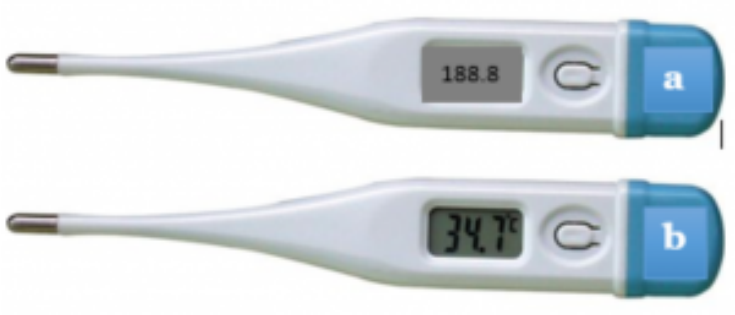


Figure 3. Standard digital thermometer used in laboratory.

Measuring the Tympanic Temperature

As with the oral thermometer, clean the probe of the tympanic thermometer with alcohol before and after use. Cover the probe with a plastic tympanic probe tip cover. Gently insert the probe into the ear canal until the canal is sealed.

You will record three replicates of the tympanic temperature. Wait at least one minute between replicates to allow the ear canal temperature to return to normal

after being in contact with the probe. Use the formula to convert between °F and °C if necessary and calculate the mean.

Part 2. The Pulse

When left ventricle of the heart contracts and ejects blood into systemic circulation, waves of blood press on the walls of the arteries, causing a pulsation. This pulsation can be detected manually when the arteries are sufficiently close to the surface of the skin. For example, the pulse can be detected on either side of the neck, at the carotid arteries, or on the radial artery at the wrist. The normal pulse rate for a healthy adult is 60-100 beats per minute but an elite athlete may have a resting pulse range from 40-60 beats per minute.

Figure 4. Taking the radial pulse. Using the index and middle finger, lightly press upon the radial artery and count the number of pulsations per minute. Image CC BY-SA 3.0.

Figure 4. Taking the radial pulse

Measuring the pulse

- Using the first and second fingertips, press firmly but gently on the **radial artery** until you feel a pulse.
- Count pulsations for 60 seconds (or for 15 seconds and then multiply by four to calculate beats per minute).
- When counting, do not watch the clock continuously, but concentrate on the beats of the pulse.

- **Complete three total replicates, recording the pulses on your lab report. Calculate the mean of the three pulse readings.**

Part 3. Respiratory Rate

The normal respiratory rate for a healthy adult at rest is 12-16 breaths per minute.

You will count the number of breaths a person takes in one minute. This should be done when the person has been at rest for at least five minutes. Simply watch the rise and fall of a person's chest when the person is not aware that you are taking their respiratory rate. It is not unusual to get erroneous resting respiratory rates when the person knows you are assessing their breathing.

- **Count the respiratory rate** of your lab partner once they have been in the seated resting position for more than five minutes.
- Count the rise and fall of their chest or shoulders for 30 seconds and multiply that number by 2.
- **Conduct three total trials and record the mean.**

After assessing respiration, use the stethoscope to **perform auscultation of your partner's lung sounds**. Listen to your partner's lungs through their shirt. Have the student inhale and exhale deeply through the mouth. Listen at each site indicated in Figure 5.

- Upper Right and Left Quadrant
- Lower Right and Left Quadrant

Listen for any abnormal noise (crackling or wheezing) and note it for the appropriate quadrant. **Record your findings on your lab report.** If you hear no noise, chart the lung sounds as clear in all quadrants.

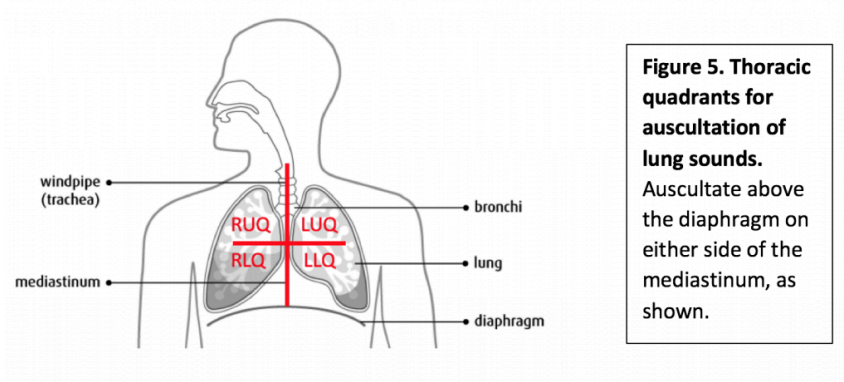


Figure 5. Thoracic quadrants

Part 4. Blood Pressure

Blood Pressure (BP) measurement indicates the 1.) pressure in peripheral arteries when the left ventricle of the heart is pumping blood to systemic arteries (the systolic pressure) and 2.) the pressure in the systemic arteries when the heart is relaxing and filling with blood (the diastolic pressure).

Blood pressure is reported as systolic pressure over diastolic pressure, the units (mm Hg) cancel each other out and are not reported. Normal healthy adult Blood Pressures should have the systolic pressure below 120 mmHg and the diastolic pressure between 70-80 mmHg. Remember the units (mmHg) cancel each other out and are not recorded.

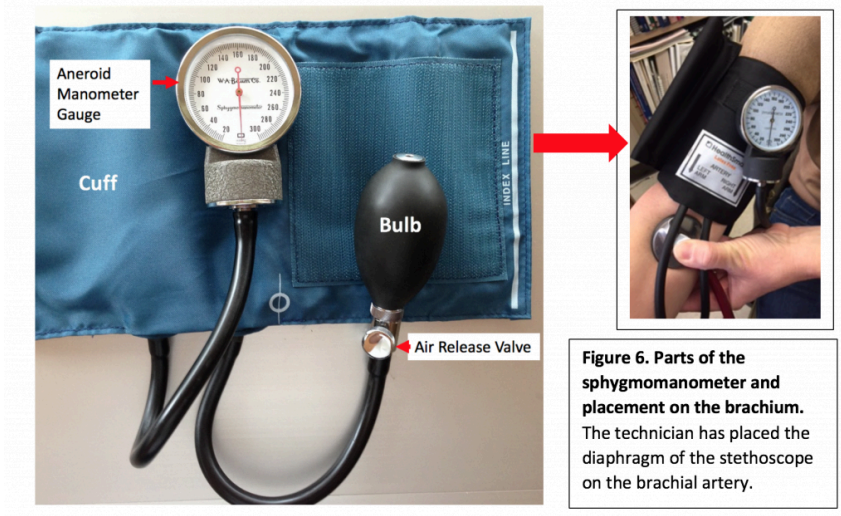


Figure 6. Parts of the sphygmomanometer.

How to take a blood pressure using the sphygmomanometer and stethoscope:

- Before beginning obtain a blood pressure cuff (sphygmomanometer) and a stethoscope. Wipe the diaphragm and the earpieces of the stethoscope with an alcohol pad.
- Place the cuff on the brachial area of the arm with the lower edge above the cubital fossa.
- Align the artery label on the cuff with the brachial artery.
- Wrap the cuff tightly around the arm, so it doesn't slide around.
- Rest the subject's arm on the table with the cuff at the same level as the subject's heart.
- Palpate the brachial artery for correct placement of the diaphragm of the stethoscope.
- Put on the stethoscope and place the diaphragm of the stethoscope on the brachial artery.
- Place your thumb on the head of the stethoscope and your fingers on the posterior aspect of the subject's arm to secure the stethoscope in the proper position.

- Make sure the Aneroid Manometer Gauge is clipped securely to the gauge holder.
- With your free hand, secure the air release valve enough to inflate the cuff without any air escaping.
- Inflate cuff by squeezing the inflation bulb while watching the needle in the Aneroid Manometer Gauge advance to about **160 mmHg**. No sound should be heard through the stethoscope.
- Stop squeezing the inflation bulb and start to slowly release air from the cuff by turning the air release valve with your thumb and index finger.
- **Listen for the first sound, the "Sounds of Korotkoff"** which is the turbulence made by the blood as it flows through the brachial artery again once the pressure of the cuff decreases.
- The first Sound of Korotkoff is a "tapping" sound. **Make note of the number on the Aneroid Manometer Gauge at which you first heard this sound. This is the Systolic Pressure.**
- Continue to slowly deflate the cuff. You will hear the sound of the turbulence decrease until you no longer hear any sound. **Note the number on the Aneroid Manometer Gauge when you no longer heard any sound and record this as the Diastolic Pressure.**
- Make sure the cuff is fully deflated and record the BP measurement in the lab report.
- Retake the BP two more times on the same arm making sure the equipment remains in the same position and the arm is at the same level of the heart. Wait a few minutes in between measurements, and make sure cuff is fully deflated in between trials. **Record the three measurements in the lab report, and calculate the mean of the three trials.**

Part 5. Body Mass Index

Remove your shoes, and go to the wall in your lab room that has the tape measure taped to the wall. Measure your height with your lab partner holding a 12-inch ruler or pen at the top of your head making sure the ruler or pen is parallel with the floor.

- Record your height in cm
- Have your height in centimeters ready before going to the scale

- Follow the posted instructions on how to use the laboratory scale
- Please give students using the scale privacy while recording their data

Imperial English BMI Formula: weight (lbs) x 703 / height (in^2)

Metric BMI Formula: weight (kg) / height (m^2)

Don't forget: Download and print your lab report from Canvas. Take your lab report to lab to complete the exercise.

Sources for this exercise

- Haen, K.M. Biology 256 lecture. Spring 2017.
- Sund-Levander M, Forsberg C, Wahren LK. Normal oral, rectal, tympanic and axillary body temperature in adult men and women: A systematic literature review. *Scandinavian Journal of Caring Sciences* 2002; **16**: 122–128.
- Stanfield, Cindy L., and William J. Germann. *Principles of Human Physiology*. Third ed., San Francisco, Pearson, 2007, pp. 13-15.
- Fox, S. I. (2013). *Human Physiology* (13th ed., pp. 7-8). New York, New York: McGraw Hill.
- Krumhardt, Ph.D.,M.T. (ASCP), B. (2013). *Exercises in Human Physiology with Personal Health Assessment* (Revised ed., p. 18). Redwood City, CA: Star Publishing Company, Inc.
- Dehghan, M., & Merchant, A. T. (2008, September 9). Is bioelectrical impedance accurate for use in large epidemiological studies? *Nutrition Journal, 7*(26). https://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-7-26

Electronic Resources

- Hopkins Medicine: Vital Signs (Body Temperature, Pulse Rate, Respiration

Rate, Blood Pressure) Last accessed June 26, 2017.

- Mayo Clinic: Pulse pressure: An indicator of heart health? Last accessed June 26, 2017.

INTRODUCTION TO DATA ACQUISITION IN HUMAN PHYSIOLOGY

Introduction to iWorx

This week's activity will teach you the basics of how to use the hardware and software that will help you acquire data for most of the physiology labs this semester. This activity uses a **pulse plethysmograph**, a sensor that records and measures a subject's pulse waves, quantifying the sensation you felt when physically taking a pulse with your two fingers over an artery in last week's lab.

Key Points to Remember for this Week's Lab

The data acquisition hardware used in 256L is **iWorx**, and the software that interprets raw iWorx data is called **LabScribe**.

Turn off and put away all personal electronic devices before starting any experiment.

For the first lab, your research team should create a folder on the desktop of the lab computer to keep your group's files in.

Sensors should be secured to the body with belts snug, but not too tight to prevent blood flow or compress underlying tissues. Subjects should sit **as quietly and motionless as possible** while the device is recording.

Click **Record** to begin taking data. As soon as you start recording, type the

subject's name in the **Mark Box** to denote the subject's data. Click **Stop** to quit recording data.

Save **.iwxdata files** to your folder with a descriptive name, so you can find the data later.

For all Biol 256L experiments, students will work in assigned lab groups (your 256L research team). During experiments, each student will record his or her own data by taking turns serving as the experimental subject. To fully participate, students should rotate through the following roles:

- Subject
- Student(s) helping the subject
- Reader of experimental methods
- Computer operator

PART I. Preparing for the lab

To Open Labscribe and begin an experiment, follow this Set-Up sequence each time you begin a Lab Experiment. **Make sure each step is completed in order.**

- **Turn On iWorx Unit Box** on Lab Table at the switch at the back of the box. Make sure **Green Light** is **lit** and all equipment is attached to the iWorx box.
- Log into the computer using your ISU ID.
- Click the folder icon in the lower left task bar.
- Click "this PC" in the left side task bar.
- Double click the Biol 256L Course Materials P-Drive under Network Locations.
- Double click the "Week2_PulsePlethysmograph" file.
- Click OK to the Hardware Found Box, if it appears.

After the experiment file is opened, the below screen will appear *(Figure 1)*.

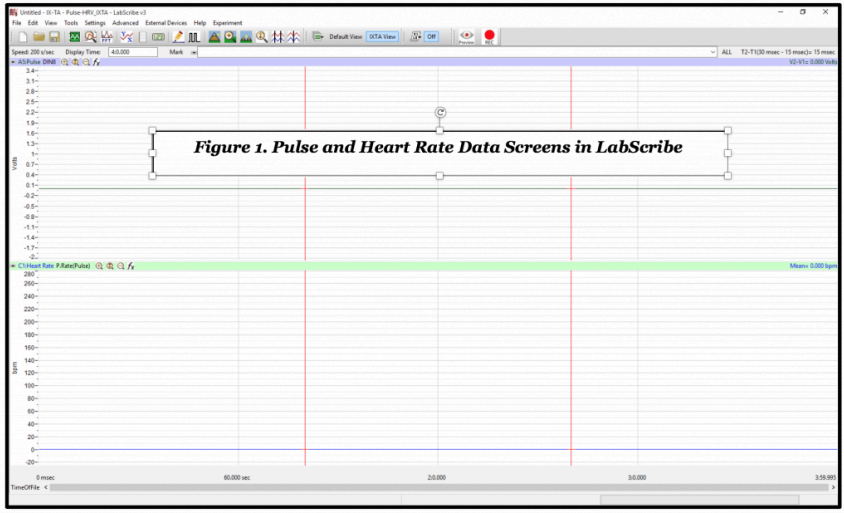


Figure 1. Pulse and heart rate data screens in LabScribe

The pulse plethysmograph is the iWorx sensor that you will use in this activity. It records the arterial pulse in a finger. The data on the Labscribe screen should look like smooth waves, representing the cardiac cycle. If you get a lot of inconsistent, messy waves, the sensor may not be appropriately attached, or the subject may be moving.

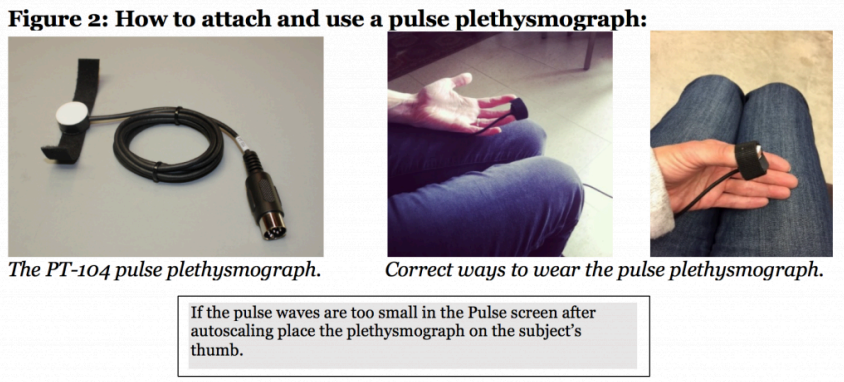


Figure 2. How to attach and use a pulse plethsmograph

PART II. Placing the Pulse Plethysmograph

- **Pick the experimental subject.**
- **Snugly velcro the plethysmograph with the white, flat side facing the finger pad on the middle finger or thumb. It should be snug!,** but should not be so tight to compress arteries and diminish the signal.
- **Subject should sit away from the table, quietly, with palms up, in his or her lap.** The subject (and attached wires) should not be touched or moving.
- **Check to make sure personal electronic devices are off and put away.**

PART III: Recording Data and Adding Marks

How to make Marks on a Recording for Noting Who the Data Belongs to in the Data File:

Large amounts of data can be recorded with LabScribe software, but, to be useful, the data that is interesting needs to be located and retrieved easily. Most often, data from multiple people (you and your lab team members) will all be recorded in the same file. Specific sections of data can be located or distinguished from other sections by placing marks and comments at those locations in the data file. Marks, with or without text comments attached, can be placed on the data during or after the recording.

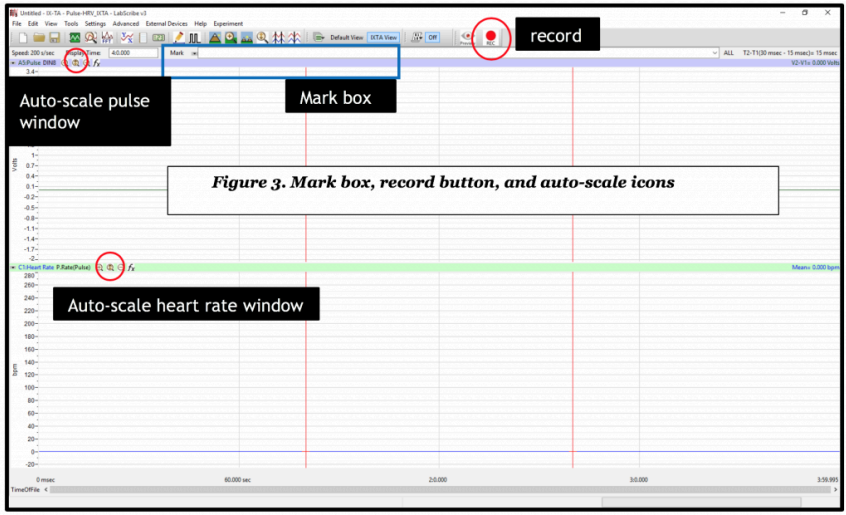


Figure 3. Mark box, record button, and auto-scale icons

To practice adding marks with comments during a recording, follow these instructions:

- Click Record (*Figure 3, above, Large Red Circle.)*
- If necessary, **adjust the height of the traces on the Pulse and Heart Rate channels by using the AutoScale buttons** (*Figure 3 Small Red Circles)* to help you see the data better.
- During data recording, an active text cursor appears in the **Mark Box** (*Figure 3 Blue box*). **Type the subject's name in the Mark Box using the keyboard.**
- **Press the Enter key on the keyboard** to place the mark on the recording. The wording typed in the Mark box will appear in the lower right-hand corner of the data screen and move along with the subject's data.
- **Record for 60 seconds** once the pulse wave pattern in the upper window looks like a regular (periodic) wave.
- After collecting normal data for 60 seconds, **click on the Stop button to end the recording.** Your recording should look similar to the example data screen in *Figure 4.*

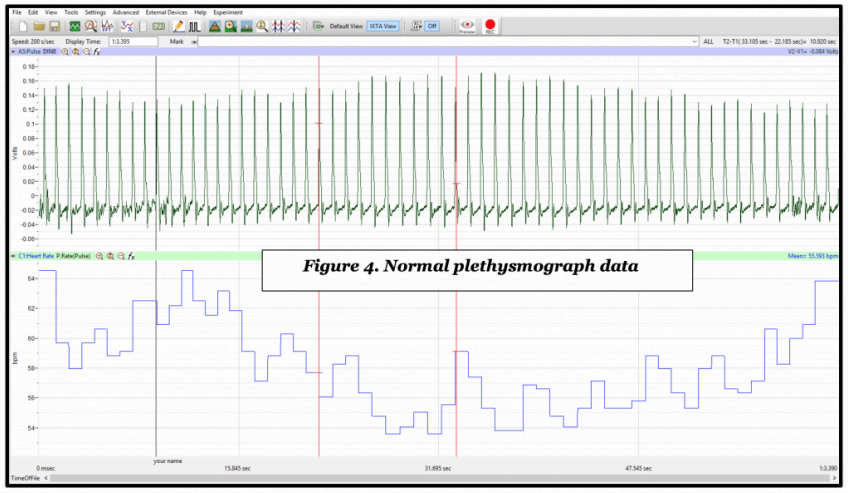


Figure 4. Normal plesmograph data

PART IV: Saving your Data

Open the File menu and Select **Save As** in the File menu, type a name for the file. Choose a destination on the computer to which to save the file, like a lab group folder on the Desktop). Designate the file type as Your Lab section # and your Table # .iwxdata. Click on the Save button to save the data file **(Ex. Section4Table2.iwxdata).**

To save a file:

- **Open the File menu and click "Save As."**
- Enter a descriptive **file name.**
- **Save file** to your group's folder.
- **Next, help others serve as the subject.**

Go to the **Week 2 Lab Report** you downloaded or printed from Canvas and follow the instructions for completing your portion of the activity. Once your own data is gathered, another student will be the subject of this activity and others at the table will rotate duties.

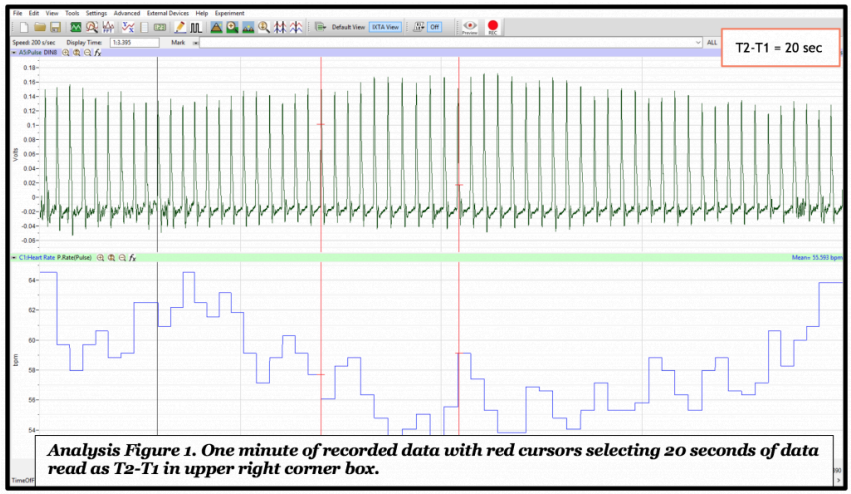
Note: you may want to record all the subjects' pulse data before helping each other do the data analysis on the lab report, but remember to correctly **MARK EACH SUBJECT'S DATA**, so you can find it later!

IMPORTANT! The following analysis must be completed in LabScribe in order to fill out the Week 2 Lab Report. You must save your data!

PART V. Data Analysis

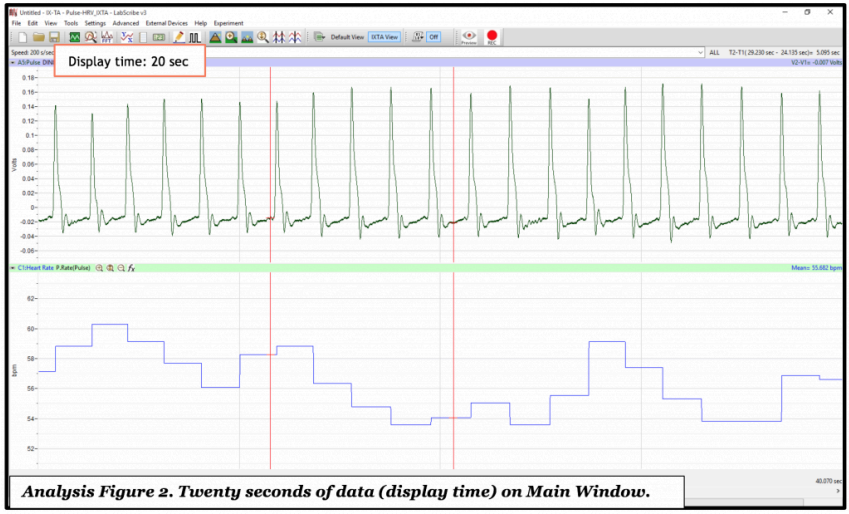
Note: you may hover over LabScribe software icons to see the function of each button.

- Find a 20 second portion of your Pulse data. To do this click on **Double Display Time**.
- Click on the **Two Cursors** mode and move the cursors until you see that you have 20 seconds listed as T2-T1 on the top right tool bar (*Analysis Figure 1, below).*



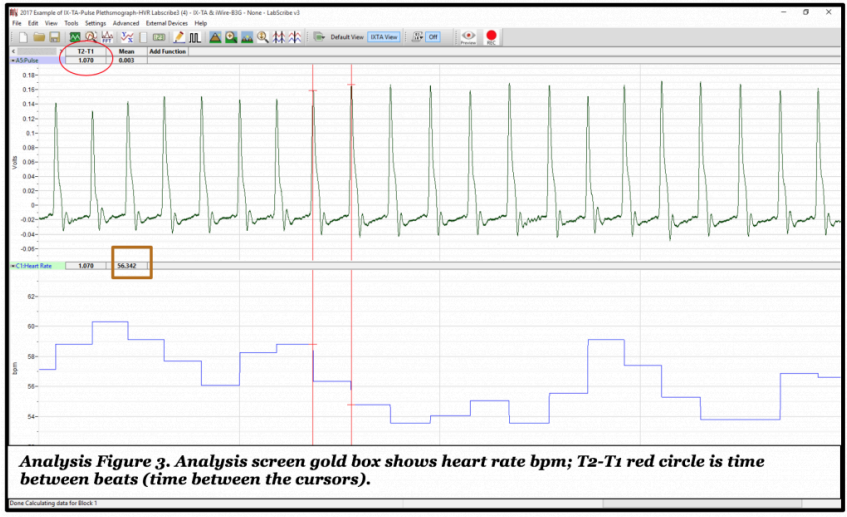
Analysis Figure 1. One minute of recorded data with red cursors selecting 20 seconds of data read as T2-T1 in upper right corner box.

- Next, click on the **Zoom Between Cursors** icon. Now the segment of pulse waves on your screen is just 20 seconds of your recording on the Main Window (see display time in upper task bar) *(Analysis Figure 2, below).*



Analysis Figure 2. Twenty seconds of data (display time) on Main Window.

- Click the **Analysis** icon (magnifying glass over a graph) to switch to the data Analysis screen.
- Next, place the first cursor at the very peak of the first wave and the second cursor on the very peak of the second pulse wave (similar to what you see for the two waves in *Analysis Figure 3*). *When the cursor is close to the peak, you may use the arrow keys on your computer keypad to move the cursor precisely to the top of the peaks.*



Analysis Figure 3. Analysis screen gold box shows heart rate bpm; T2-T1 red circle is time between beats (time between the cursors).

- In your **Lab Report Table** record the **Heart Rate in bpm** (found in the gold square in the figure) on the lower Heart Rate Screen and the **Time Between Beats** found under T2-T1 (Red Circle) on the Pulse Screen (*Analysis Figure 3, above)*.
- Now move the cursor from the first wave to the peak of the third wave, so now you have a cursor remaining on the second wave and a cursor on the third wave, record the same values for this set of data.
- Next, move the cursor from the second wave to the fourth wave, record the data. Continue moving the cursors in this manner until you have recorded data **for ten waves.**

- **Calculate the mean for the 10 waves on your lab report and answer the questions.** You're all done with iWorx for this week!

PROPERTIES OF BLOOD AS A BUFFER AND BLOOD GLUCOSE

Biol 256 Week 3 Laboratory

Karri Haen Whitmer

Part I: Blood pH Homeostasis

Acidity and alkalinity describe a property of chemicals based upon relative concentration of hydrogen ions in a solution. The pH scale measures this value and ranges from 0 to 14. A pH of 7.0 is considered neutral. A pH value greater than 7 is basic, and a pH less than 7.0 is acidic.

The pH scale is defined as the negative log of the hydrogen ion concentration of a solution (figure 1). This means each pH value on the scale represents a ten-fold difference in hydrogen ion molarity than the next value. For instance, a solution with pH of 8.0 is one-hundred times *more basic* than a solution with a pH of 6.0. Similarly, a solution with a pH of 5.0 is ten times *more acidic* than a solution with a pH of 6.0.

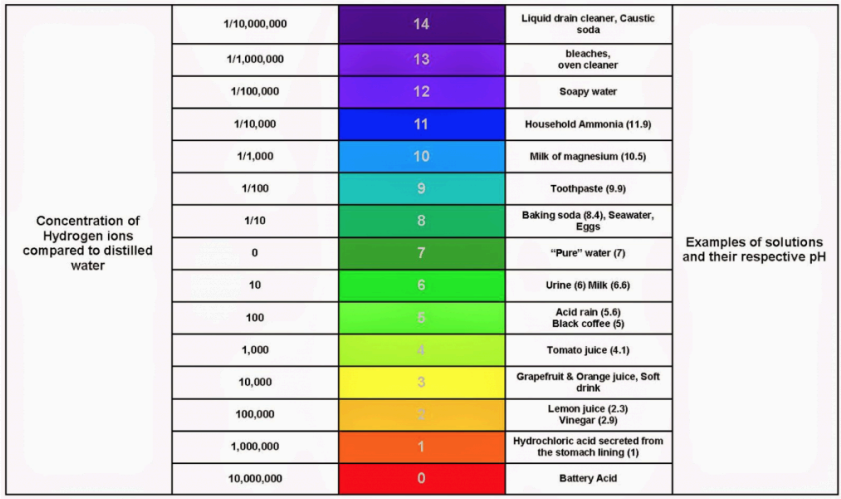


Figure 1. pH scale showing relative hydrogen ion concentrations. Examples of solutions with their pH listed, and concentration of Hydrogen ions compared to distilled water. For comparison, other biologically relevant solutions include the pH of pancreatic juice, which is 8.8, and seminal fluid, which has a pH of 7.8. By ChemEd DL (pH Scale)/CC-BY-SA.

In biological systems, it is important to keep the pH of a solution within a narrow range of values. To accomplish this, buffers are used. Buffers resist changes in the pH of a solution when hydrogen ions or hydroxide are added (or removed). Buffers dissociate in solution and neutralize extra hydrogen ions or hydroxide ions by participating in reactions with them.

Normal blood pH is 7.4, and arterial pH may only vary between 7.35 and 7.45 without being pathological. The Carbonic Acid-Bicarbonate buffer system is the most important buffer for maintaining the pH homeostasis of blood. In this system, gaseous metabolic waste carbon dioxide reacts with water to form carbonic acid, which quickly dissociates into a hydrogen ion and bicarbonate (see below).

$$\mathbf{CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H+ + HCO_3-}$$

Besides the carbonic acid-bicarbonate buffer system, there are other buffers in whole blood, including the phosphate buffer system. Additionally, some proteins have buffering capacity, such as hemoglobin and blood serum albumin (a common carrier

protein in blood). These have a lesser effect than the carbonic acid-bicarbonate system on maintaining blood pH homeostasis.

In this week's activity, you will test whether five solutions behave as buffers:

- **Saline** (a salt water solution)
- **Phosphate Buffered Saline (PBS),** which is a solution of disodium hydrogen phosphate, sodium chloride and potassium chloride commonly used the lab
- **5% blood serum albumin** (a protein found in blood serum)
- **Blood Plasma** (Sheep), **diluted 10X with PBS** (the liquid part of blood, which contains all the water, ions and proteins minus any formed elements)
- **Whole Blood (Sheep) diluted 10X with PBS** (containing all the components in "live" blood, including the cells)

Part II: Blood Glucose Homeostasis

Blood sugar homeostasis is important 1.) to consistently provide body cells with the necessary glucose to sustain metabolic reactions and 2.) to protect cells against damage that can occur when circulating blood glucose levels are too high for too long. For healthy individuals, the American Diabetes Association recommends a fasting (no food or drink, except water, for 8 hours) plasma glucose level of less than 100 mg/dL and, 2 hours after meals, a concentration less than 140 mg/dL.

In order to tightly control blood glucose levels, α- and β-cells of the endocrine pancreas secrete two hormones – insulin and glucagon. When eating a meal, the surge in blood sugar is detected directly by receptors on the pancreatic islet β-cells. These cells then secrete insulin, which binds to insulin receptors on various body tissues. Occupied insulin receptors signal body cells to increase the number of glucose transporters, special proteins that shunt glucose from the blood, through cell plasma membranes, and into the cell. The increase in transporters results in quick internalization of blood glucose by the cells.

When blood glucose levels are too low, e.g., less than 70 mg/dL or lower, the body may not have enough circulating blood glucose to sustain the body's chemical reactions. Symptoms of low blood sugar (hypoglycemia) can be severe and include:

nervousness, shaking, fatigue, blurry vision, trouble thinking, and even the loss of consciousness.

Low blood sugar is detected by the α- cells of the pancreas, which increase glucagon secretion. Glucagon activates liver enzymes, which then convert the glucose storage molecule, glycogen, into glucose. Glucagon also initiates the endogenous synthesis of glucose from other biomolecules like amino acids. The newly released or synthesized glucose passes into the blood stream and raises blood glucose levels.

Diabetes is a condition in which the body fails to respond to insulin, resulting in the body's inability to control glucose homeostasis. Typically, in Type I diabetes, pancreatic β-cells are destroyed by an autoimmune reaction resulting in low or absent insulin in the body. Type II diabetes is also called "insulin resistance." Generally, in this disease, the body manufactures insulin, but there is a problem that prevents insulin receptors from sending signals that increase the presence of glucose transporters in the plasma membranes of cells.

In Lab this week, your TA may test your Blood Glucose using a Contour Blood Glucose Monitoring System. A blood sample will be obtained from the side of your middle or ring finger using an Accu-Chek Safe-T-Pro Lancet. It delivers a quick, almost painless pinprick and only a small amount of blood is needed.

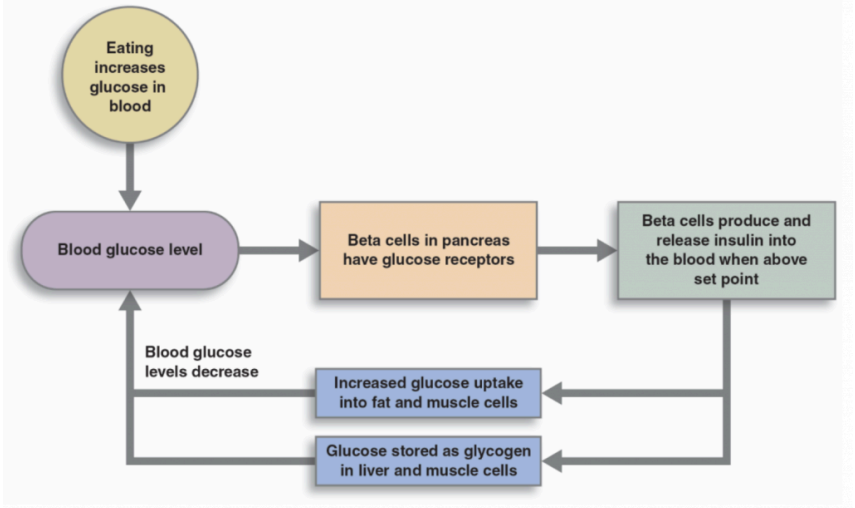


Figure 2. Blood glucose homeostasis. Figure shows pancreatic signaling in response to high blood sugar. Figure courtesy of Anatomy & Physiology (Open + Free) CC BY-NC 4.0.

Laboratory Methods

Exercises

The following text describes the laboratory methods for analysis of blood buffering and blood glucose.

In this experiment, students will determine the buffering capabilities of a variety of solutions by measuring the pH of the solutions when they are treated with a weak base. Each group of students will measure the pH changes that occur in Saline,

Phosphate Buffered Saline (PBS), 5% Albumin in PBS, Blood Plasma diluted 1/10 with PBS, and Whole Blood diluted 1/10 with PBS.

Laboratory Objectives

- Students will practice pipetting technique.
- We will use iWorx and calibrate the electrodes with LabScribe.
- The calibrated electrodes will be used to determine the pH changes that occur with addition of NaOH to the test solutions.
- Analyze the change in pH for each solution and determine if it is a buffer.
- Finally, we will measure each student's blood glucose level with a blood glucose monitor.

Equipment Required

- PC Computer
- iWorx TA (IXTA) data acquisition unit
- ISE-100 combination pH electrodE
- Saline
- PBS
- 5% albumin in PBS
- 1/10 blood plasma in PBS
- 1/10 whole blood in PBS
- Beakers
- 10-100 µL or 20-100 µL pipette
- Distilled water
- Kimwipes®
- 0.1 M NaOH

Safety Precautions! Students working with solutions in this lab must wear safety googles and gloves.

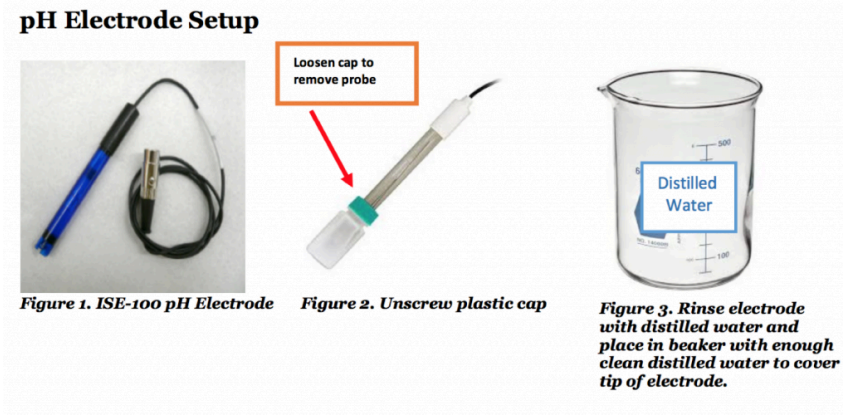
Practice using the micropipette

To draw up a specific amount of a solution, set the dial on the micropipette to the appropriate volume, then depress the plunger, and immerse the pipette tip in the solution. The micropipette plunger has a first "soft" stop. When the plunger is pushed to the first stop, it allows the pipette to draw up the programmed amount as the plunger is gently returned to the original position. To discharge the drawn-up solution, the plunger is pushed all the way down to the second "hard" stop.

To practice, place a plastic tip on the pipette, push the plunger to the first stop, submerge the pipette tip into the distilled water and gently allow the plunger to return to its original position. Look at the plastic tip and note how much distilled water has been taken into the tip. Discharge the distilled water back into the container of distilled water and repeat. Did you get the same amount of distilled water drawn into the tip as the first time? Continue practicing until you feel confident that you are operating the pipette properly.

PART I. Set up the computer

- Turn On iWorx Unit Box on Lab Table at the switch at the back of the box. Make sure Green Light is lit and all equipment is attached to the iWorx box.
- Log into the computer using your ISU ID.
- Click the folder icon in the lower left task bar.
- Click "this PC" in the left side task bar.
- Double click the Biol 256L Course Materials P-Drive under Network Locations.
- Double click the "Week3_BiologicalBuffers" file.
- Click OK to the Hardware Found Box, if it appears.



Methods figures 1-3. The ISE-100 pH electrode and associated materials.

PART II: Calibrate the pH Electrode

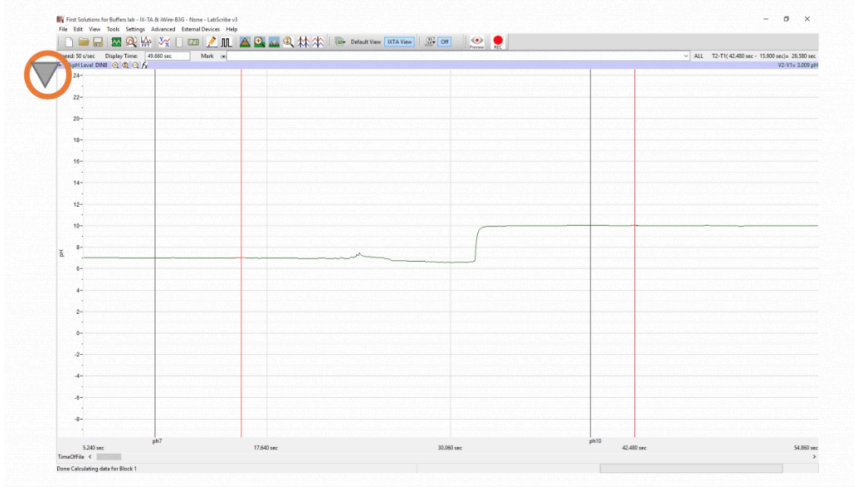
Note: electrodes are delicate and expensive sensors, which can easily crack. Do not tap or drop it. When placing it in a beaker, do so carefully.

- **Remove the ISE-100 pH electrode from its bottle** of buffer by unscrewing the cap a little so that you can easily pull out the electrode. Make sure top portion of the bottle is removed.
- Replace screw cap of bottle if totally separated and place back in dry storage beaker where you found the electrode. **Rinse the electrode with distilled water** while holding the electrode over the large beaker used for the collection of **waste liquids**.
- **Place the tip of the ISE-100 pH electrode in a small beaker** containing enough room temperature distilled water to submerge the tip. Keep the electrode in distilled water for a few seconds.
- **Obtain the calibration buffers:** One beaker will contain pH 7 buffer and the other will contain pH 10 buffer. Each beaker should be filled with enough buffer to cover the tip of the ISE-100 pH electrode, but **NO MORE is necessary!**
- **Remove the electrode from the distilled water** and gently blot any extra drops of water using Kimwipes. Submerge the end of the electrode in the beaker of pH 7 buffer.

- **Click Record** (the red button on the upper toolbar) on the LabScribe Main window to begin recording. After a few seconds, the trace will reach a stable baseline. Type pH 7 in the Mark box to the right of the Mark button (at the top- center of the screen). Press the Enter key on the keyboard to mark the stable baseline of the recording. This will mark the output of the ISE-100 pH electrode in pH 7 buffer. You may continue recording while changing the beakers of buffers.
- **Remove the ISE-100 pH electrode from the beaker of pH 7 buffer.** Hold the electrode over the beaker used for collecting waste liquid and rinse it with distilled water. Blot any extra drops of water with Kimwipe.
- **Position the electrode in the beaker of pH 10 buffer.**
- As you continue to record, the trace will reach a stable baseline, allow it to record for several seconds. Type pH 10 in the Mark box. Press the Enter key on the keyboard to mark the stable baseline of the recording. **Click Stop on the LabScribe Main toolbar to stop recording**.
- **Remove the electrode** from the beaker of pH 10 buffer. Hold the electrode over the beaker used for collecting waste liquid, and **rinse it with distilled water** from a wash bottle. Blot any extra drops of water and **place the electrode in a clean beaker of room temperature distilled water.**

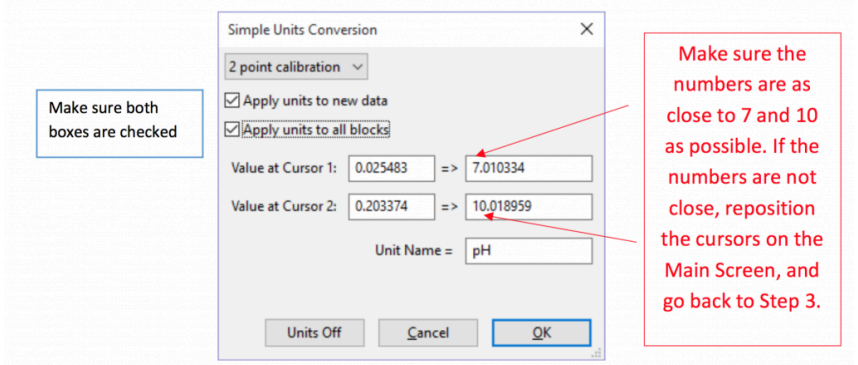
Units Conversion

- Scroll to the beginning of the calibration data for the ISE-100 pH electrode.
- To show the data collected at pH 7 and pH 10 on the Main window at the same time, you can use either the **Double Time Display** icon to adjust the **Display Time**. **Click Double Time several times until your pH 7 mark and the pH 10 mark are on the same screen** or the Double Cursor icon will allow two red cursors to appear on the Main window.
- Place one **red cursor** on a flat section of data collected when the ISE-100 pH electrode was in the **pH 7 buffer** and the **second red cursor** on a flat section of data collected when the electrode was in the **pH 10 buffer**.
- Click on the **Auto Scale** icon in the tool bar above the graph screen.



Methods figure 4. pH calibration data recorded showing the positions of the red cursors for changing the recorded voltage to pH values. Note gray triangle in orange circle.

- To convert the voltages at the positions of the cursors to pH values, use the **Simple Units Conversion dialogue window**. To access this dialogue window, **click on the grey arrow** (the upper left corner of the screen, see orange circle on figure 4, above) to the left of the channel title, pH, to open the channel menu.
- Select **Units** from the **channel menu**, and select **Simple** from the **units submenu**.
- On the **Units Conversion** window, (see picture below) make sure **2 point calibration is selected** in 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 the two buffers used in the calibration recording in the corresponding boxes on the right side of the conversion equations.** Enter the name of the units, pH, in the box below the buffer values.
- **Click on the OK button** in the lower right corner of the window to activate the Units Conversion.



Methods figure 5. Simple units conversion dialog window showing pH units conversion settings.

PART III: Test the Potential Buffers

Important: Click on the single-cursor mode icon for the rest of this experiment.

Solutions to test: Saline, Phosphate Buffered Saline (PBS), 5% Bovine Serum Albumin (BSA) in PBS, 10X sheep blood plasma in PBS, 10X sheep whole blood in PBS

For each solution to be tested:

- Click on the **single cursor mode icon.**
- Place approximately **10mL of the test solution** in a small beaker. **Label each buffer solution** with a removable paper label which you will remove during clean up at the completion of the lab.
- Immerse the electrode in the test solution and **click Record**. Wait a few minutes for the pH to stabilize and mark this point as the Start for Solution X (name the solution) press ENTER.
- Add approximately **50?L of NaOH** to the test solution. Gently swirl the solution and wait a few seconds for the pH to stabilize. **Mark this point as 1 aliquot.** Press ENTER.
- If the pH has changed less than 0.5 units, add another 50?L of NaOH and mark 2 aliquots and press ENTER once the solution has stabilized. Continue

adding 50?L of NaOH and marking until you observe a change of 0.5 units or greater. **Stop adding NaOH if you have added 15 aliquots of 50?L without a change in pH of at least 0.5 units.**

- In **Data Table 3.1 of your Lab Report**, record the number of 50?L aliquots of NaOH required to cause the pH to go up at least 0.5 pH units. If you observed no change in pH after 15 aliquots were added, note "15+" in the data table.
- Repeat the procedure with the other test solutions making sure to **rinse the electrode** with clean distilled water and blotting it dry with a **clean Kimwipe** between solutions.
- Once you have tested all the test solutions, rinse the electrode again with distilled water and blot it with a Kimwipe. Gently insert the electrode into its original bottle of buffer and gently screw on the cap. **Make sure the electrode is submersed in the buffer and stays upright! If the electrode dries out the equipment will be damaged. Please see the below figure.**
- Finish the Week 3 Properties of Blood Worksheet.
- **Note: Because the plasma and whole blood are diluted tenfold, their buffering ability is diluted as well.** Therefore, assume these were ten times less effective than if undiluted plasma or blood were used.

Lab Clean Up

When the buffer part of lab is complete, please **remove small labels from beakers** and place in the trash. **Rise the beakers several times with water** and return them to their original locations to dry. Please leave the electrode in the same condition is was found: **place electrode back in its small beaker with lid on** (please make sure buffer solution is in the container because the electrodes must not dry out), and **place the electrode storage bottle upright inside a larger beaker**.

Blood glucose testing

When buffer work in lab has completed, TAs will offer students the opportunity to participate in a blood glucose test. Students will record their last meal and blood glucose value on the lab report; however, this part of the lab is volunteer only, since students must draw blood. TAs may offer one point extra credit for participation in

this activity. Students who decide to participate must sign the consent form at the TA podium.

BODY TEMPERATURE HOMEOSTASIS

Cold Stress and the Cold Pressor Test

Maintaining homeostasis requires that the body continuously monitor its internal conditions. From body temperature to blood pressure to levels of certain nutrients, each physiological condition has a particular set point. A **set point** is the physiological value around which the normal range fluctuates. A **normal range** is the restricted set of values that is optimally healthful and stable. For example, the set point for normal human body temperature is approximately 37°C (98.6°F). Physiological parameters, such as body temperature and blood pressure, tend to fluctuate within a normal range a few degrees above and below that point. Control centers in the brain and other parts of the body monitor and react to deviations from homeostasis using negative feedback. **Negative feedback** is a mechanism that reverses a deviation from the set point. Therefore, negative feedback maintains body parameters within their normal range. The maintenance of homeostasis by negative feedback goes on throughout the body at all times.

The human body regulates body temperature through a process called thermoregulation, in which the body can maintain its temperature within certain boundaries, even when the surrounding temperature is very different. The core temperature of the body remains steady at around 36.5–37.5 °C (or 97.7–99.5 °F). In the process of ATP production by cells throughout the body, approximately 60 percent of the energy produced is in the form of heat used to maintain body temperature. Thermoregulation is an example of negative feedback.

The hypothalamus in the brain is the master switch that works as a thermostat to regulate the body's core temperature (Figure 1). If the temperature is too high, the hypothalamus can initiate several processes to lower it. These include increasing the circulation of the blood to the surface of the body to allow for the dissipation of heat through the skin and initiation of sweating to allow evaporation of water on the skin to cool its surface. Conversely, if the temperature falls below the set core

temperature, the hypothalamus can initiate shivering to generate heat. The body uses more energy and generates more heat. In addition, thyroid hormone will stimulate more energy use and heat production by cells throughout the body. An environment is said to be thermoneutral when the body does not expend or release energy to maintain its core temperature. For a naked human, this is an ambient air temperature of around 84 °F. If the temperature is higher, for example, when wearing clothes, the body compensates with cooling mechanisms. The body loses heat through the mechanisms of heat exchange.

Mechanisms of Heat Exchange

When the environment is not thermoneutral, the body uses four mechanisms of heat exchange to maintain homeostasis: conduction, convection, radiation, and evaporation. Each of these mechanisms relies on the property of heat to flow from a higher concentration to a lower concentration; therefore, each of the mechanisms of heat exchange varies in rate according to the temperature and conditions of the environment.

Conduction is the transfer of heat by two objects that are in direct contact with one another. It occurs when the skin comes in contact with a cold or warm object. For example, when holding a glass of ice water, the heat from your skin will warm the glass and in turn melt the ice. Alternatively, on a cold day, you might warm up by wrapping your cold hands around a hot mug of coffee. Only about 3 percent of the body's heat is lost through conduction.

Convection is the transfer of heat to the air surrounding the skin. The warmed air rises away from the body and is replaced by cooler air that is subsequently heated. Convection can also occur in water. When the water temperature is lower than the body's temperature, the body loses heat by warming the water closest to the skin, which moves away to be replaced by cooler water. The convection currents created by the temperature changes continue to draw heat away from the body more quickly than the body can replace it, resulting in hypothermia. About 15 percent of the body's heat is lost through convection.

Radiation is the transfer of heat via infrared waves. This occurs between any two objects when their temperatures differ. A radiator can warm a room via radiant heat.

On a sunny day, the radiation from the sun warms the skin. The same principle works from the body to the environment. About 60 percent of the heat lost by the body is lost through radiation.

Evaporation is the transfer of heat by the evaporation of water. Because it takes a great deal of energy for a water molecule to change from a liquid to a gas, evaporating water (in the form of sweat) takes with it a great deal of energy from the skin. However, the rate at which evaporation occurs depends on relative humidity—more sweat evaporates in lower humidity environments. Sweating is the primary means of cooling the body during exercise, whereas at rest, about 20 percent of the heat lost by the body occurs through evaporation.

Homeostatic Response to Environmental Temperatures

Humans have a temperature regulation feedback system that works by promoting either heat loss or heat gain. When the brain's temperature regulation center receives data from the sensors indicating that the body's temperature exceeds its normal range, it stimulates a cluster of brain cells referred to as the "heat-loss center." This stimulation has three major effects:

- Blood vessels in the skin begin to dilate allowing more blood from the body core to flow to the surface of the skin allowing the heat to radiate into the environment.
- As blood flow to the skin increases, sweat glands are activated to increase their output. As the sweat evaporates from the skin surface into the surrounding air, it takes heat with it.
- The depth of respiration increases, and a person may breathe through an open mouth instead of through the nasal passageways. This increases heat loss from the lungs.

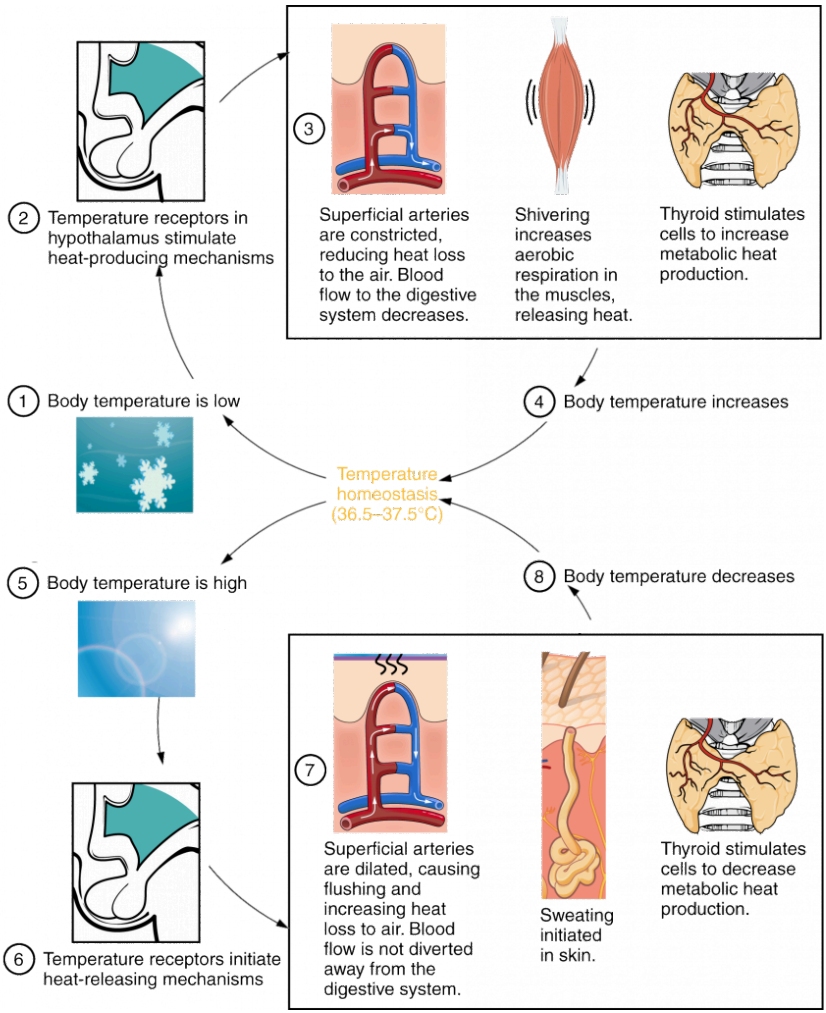


Figure 1. Hypothalamus Controls Thermoregulation. The hypothalamus controls thermoregulatory networks leading to an increase or decrease in the core body temperature. Original image OpenStax Anatomy and Physiology CC-by-4.0. Image edited by Aric Warner.

In contrast, activation of the brain's heat-gain center by exposure to cold reduces blood flow to the skin, and blood returning from the limbs is diverted into a network of deep veins (Figure 2). This arrangement traps heat closer to the body core, restricts heat loss, and increases blood pressure. If heat loss is severe, the brain triggers an increase in random signals to skeletal muscles, causing them to contract and

producing shivering. The muscle contractions of shivering release heat while using ATP. The brain also triggers the thyroid gland in the endocrine system to release thyroid hormone, which increases metabolic activity and heat production in cells throughout the body.

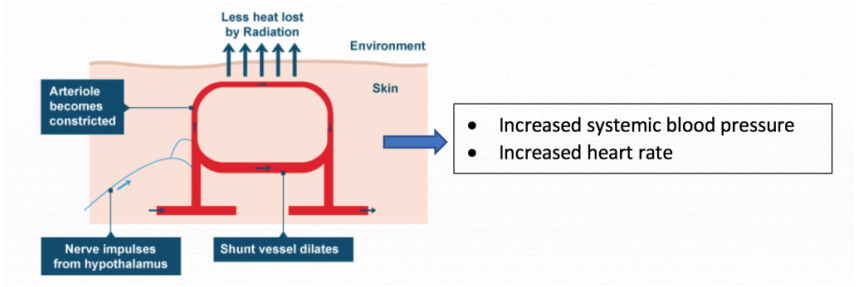


Figure 2. Physiological response to acute cold exposure. During acute cold exposure, the sympathetic nervous system releases norepinephrine, which results in vasoconstriction, increased blood pressure, and increased heart rate.

During acute exposure to cold conditions in the body:

- Activation of the sympathetic nervous system results in system-wide discharge of catecholamine (norepinephrine).
- Catecholamine causes systemic arteriolar constriction, increased heart rate and heart contractility. The heart works harder to push blood through the narrowed blood vessels.
- Constricted blood vessels in the extremities divert superficial blood flow to the body's core, thus, reducing the radiation or conduction of heat into the environment.
- Vasoconstriction increases the resistance to blood flow, and thus, increases blood pressure.
- Vasoconstriction leads to a weaker pulse (lower pulse amplitude) in the arteries of the skin, fingers and hand.

The Cold Pressor Test

Acute cold stress results in activation of the sympathetic nervous system and release of catecholamines (neurotransmitters). The release of neurotransmitter effects the cardiovascular system in a number of ways, including arterial constriction, transient tachycardia, and increased contractility of the heart. Together, these homeostatic changes result in what is called a **pressor response**, or an increase in blood pressure. The **cold pressor test** is commonly used in the clinical setting to evaluate the function of the sympathetic nervous system. In the cold pressor test, subjects immerse their hand or forearm in ice water, and their cardiovascular response is measured.

In this laboratory, we will use the cold pressor test to evaluate changes in heart rate, pulse amplitude, and arterial oxygen saturation using a pulse oximeter.

Pulse oximeters indirectly estimate the arterial oxygen saturation and report it as the oxygen saturation (SpO2) of the subject's arterial blood. SpO2 is reported as a percentage of oxygenated hemoglobin. Normal pulse oximetry values typically range from 97-100%.

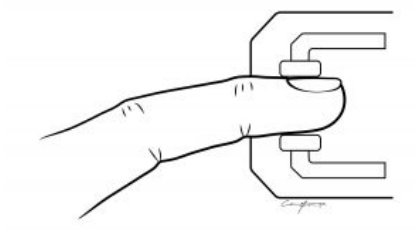


Figure 3. The pulse oximeter. Finger clamp pulse oximeters are used in the physiology laboratory. A light emitting diode rests on top of the finger, and a photodetector is located beneath the finger. Figure created by Cameron Miller CC-by-ND.

Cold pressor response experiment:

There are several hypotheses that could be testing In this laboratory. For example, we may test whether males and females have a different cold pressor response, or we may test whether the pressor response is the same in the submerged versus the non-submerged hand. After collecting the data, you will enter it into an excel file at the TA's bench for a class-wide or course-wide statistical analysis.

In preparation for lab, can you write an IF/THEN hypothesis for testing the cold pressor response in men and women?

Laboratory Methods

Exercises

In this lab you will conduct an experiment to test how acute cold exposure affects pulse amplitude, heart rate and hemoglobin-oxygen binding in men and women. You will be using a finger sensor called a pulse oximeter, which will measure the pulse as well as the peripheral arterial blood oxygenation (SpO2) in your finger.

Equipment Required

Computer

IXTA

iWire-PO2-100

Pulse oximeter

Ice bath

Heating pad

Lab activity highlights

- We will use iWorx with LabScribe to interpret pulse amplitude, heart rate and SpO2.
- **Subjects should not wear nail polish, artificial nail coverings, hand or wrist jewelry during the experiment.**
- **Subjects must wear short sleeves or sleeves that can be rolled up above the elbow.**
- **All subjects will participate in either "Baseline/Condition 1" or "Baseline/ Condition 2" but not both.**
- **All subjects will submerge their LEFT forearm in the experiments.**
- Because the pulse oximeter works by detecting

pulsation of blood vessels, subjects should sit quietly and motionless during the experiment. Other movements or vibrations could confound the pulse oximeter readings.

Getting Started

- Turn on the iWorx unit at the switch on the back of the box
- Log into your account and click the **Folder icon** in the lower left task bar
- Click "**This PC**" in the left side task bar
- Double click Biol 256L Course Materials P-Drive under "**Network Locations**"
- Double click the "**Week4_ColdPressor**" settings file
- **Place the pulse oximeter on the middle finger of the left (condition 1) or right (condition 2) hand as shown in the figure below.**
- You are now ready to start the experiment.

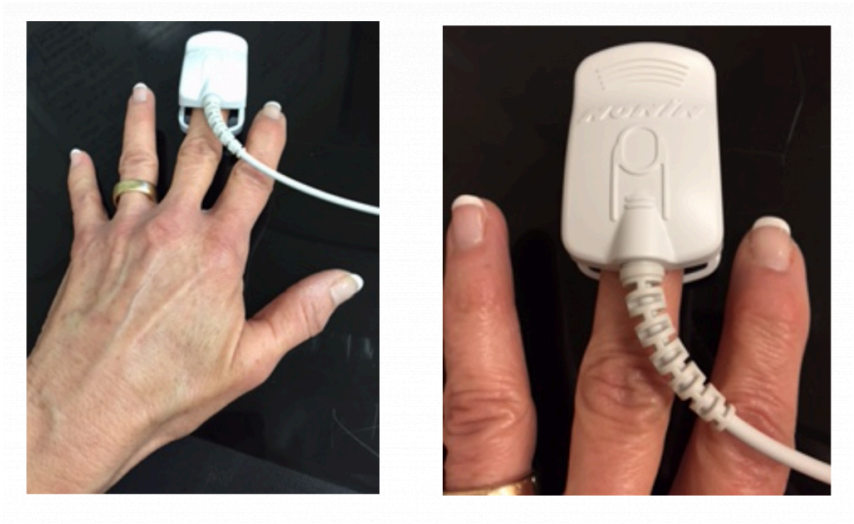


Figure 4. How to wear the pulse oximeter sensor.

EXPERIMENT: Effects of Cold Pressor Test on

Cardiovascular Functioning

IMPORTANT: This experiment requires half of the subjects to participate in Baseline/Condition 1 and half of the subjects to participate in Baseline/Condition 2. At your lab table, assign each student a condition before starting the experiment.

- **CONTROL/CONDITION 1:** Outfit the **middle finger of the left hand** with the pulse oximeter. Be prepared to submerge the left forearm in ice water at the one-minute mark.

- **CONTROL/CONDITION 2:** Outfit the **middle finger of the right hand** with the pulse oximeter. Be prepared to submerge the left forearm in ice water at the one-minute mark.

PART I. Procedure

- Check the sensor: click on the red **Record**
- Click on the **AutoScale** button at the upper task bar. Your recording should look like the traces seen below in Figure 5. If the data does not appear as shown, slightly adjust the oximeter on the finger.
- Note the location of the **Time** in the upper right corner of the window (Fig. 5b). In the figure, the time reads "one minute and twenty-two seconds." You will keep track of the time of the data recording with this timer on the Labscribe window.

Figure 5a. Example window showing correctly generated pulse, heart rate and SpO2 data.

Figure 5b. Closeup view of the pulse window showing the time as Time1 (red box).

- When the signals being recorded are suitably displayed, stop the recording and **open a new file.**
- As the subject sits quietly (without moving) **record baseline data for one minute**.
- **At exactly the one-minute mark, submerge the left forearm in the ice water.** DO NOT put the hand with instrumentation in the water. Remain as still as possible!

- Record the data for at least an additional **35 seconds** (you may record more).
- **Stop recording.**
- You may dry your arm off and warm it on a heating pad. You are done serving as subject after a single exposure to the ice bath.
- **Save the data file to the computer. Put the subject's name and Week 4 in the title.**

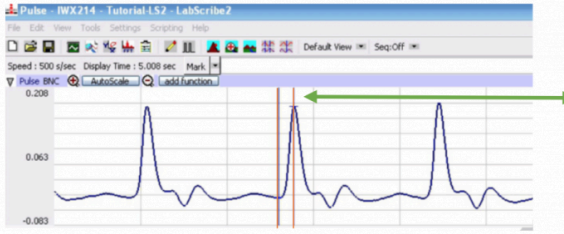
PART II. Data Analysis

This data analysis applies to both the baseline recording and to Condition 1 or 2. For baseline data, start at the very beginning of the recording and find the correct data by scrolling and using the timer on the main window.

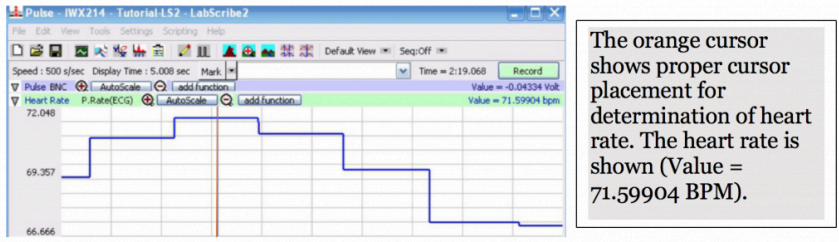
For the experimental data (condition 1 or 2), start data analysis at the 1.00 mark and scroll to 1.05 (five seconds), 1.10 (ten seconds), 1.20 (twenty seconds) and 1.30 (thirty seconds).

To begin the data analysis:

- Use the Display Time icon to adjust the Display Time of the Main window to show approximately ten complete Pulse cycles on the Main window.
- Scroll through the recording to view exemplary pulse waves at these intervals during data recording: **5 seconds, 10 seconds, 20 seconds and 30 seconds**
- Start at a pulse wave at around **5 seconds** of data recording and click the **double cursor icon** and place the cursors as follows:
- To measure the **pulse wave amplitude**, place one cursor on the baseline that precedes the pulse wave and the second cursor on the peak of the pulse wave. The value for **V2-V1** on the Pulse channel is this amplitude. Determine the pulse amplitude V2-V1 for the four pulse waves at the designated times and **record the results in your lab report**.



- To find the **heart rate**, select the **one cursor icon** and place the single cursor at the plateau of the of a heart rate trace on the **Heart Rate channel**. *See the orange cursor in the picture below.* **Record the value in BPM on your lab report for heart rate data collected at approximately 5s, 10s, 20s and 30s.**



- To find the **SpO2**, place cursor on the data at the 30 second mark of recording. Usually this line is completely flat.
- Record the **SpO2 percent**, shown on the **O2 Saturation** channel, in your lab report.

After recording the data in your lab report, open a new file for the next student.

Students may be asked to submit these data for statistical analysis:

Note: please submit your sex (M or F) and age with your data.

1. Baseline avg. heart rate
2. Baseline avg. pulse wave amplitude

3. Condition 1 avg. cold pressor heart rate
4. Condition 1 avg. cold pressor pulse wave amplitude
5. Condition 2 avg. cold pressor heart rate
6. Condition 2 avg. cold pressor pulse wave amplitude

Citations

- Some background materials adapted from *OpenStax Anatomy and Physiology,* https://openstax.org/details/books/anatomy-and-physiology. Available for free under a CC-by-4.0 license.
- OpenStax College (2013, April 25). *Anatomy and physiology*. OpenStax College. Retrieved from http://cnx.org/content/col11496/latest

CLINICAL TECHNIQUES: THE NEUROLOGICAL ASSESSMENT

Cranial Nerves

Karri Haen Whitmer

The central nervous system (CNS) consists of the brain and the spinal cord. The CNS receives sensory information from other parts of the body or the body's external environment and transmits motor information to muscles and glands by way of the peripheral nervous system (PNS). The PNS of the human includes 31 pairs of spinal nerves and 12 pairs of cranial nerves. Some nerves contain only motor nerve fibers (efferent fibers); some nerves contain only sensory nerve fibers (afferent fibers); and some nerves contain both sensory and motor nerve fibers (mixed). All spinal nerves are mixed.

Cranial nerves

The I (olfactory), II (optic), and VIII (vestibulocochlear) nerves are entirely sensory. Cranial nerves III (oculomotor), IV (trochlear), VI (abducens), XI (accessory), and XII (hypoglossal) are classified as motor, although they do contain proprioceptive afferent fibers. Cranial nerves V (trigeminal), VII (facial), IX (glossopharyngeal), and X (vagus) are mixed. All cranial nerves except the olfactory nerves are connected to the brain stem (medulla, pons, mesencephalon), and all are distributed in the head and neck except the vagus, which also supplies structures in the thorax and abdomen. Figure 1a (below) shows cranial nerves and their origins and terminations. Figure 1b shows their origins on the brain.

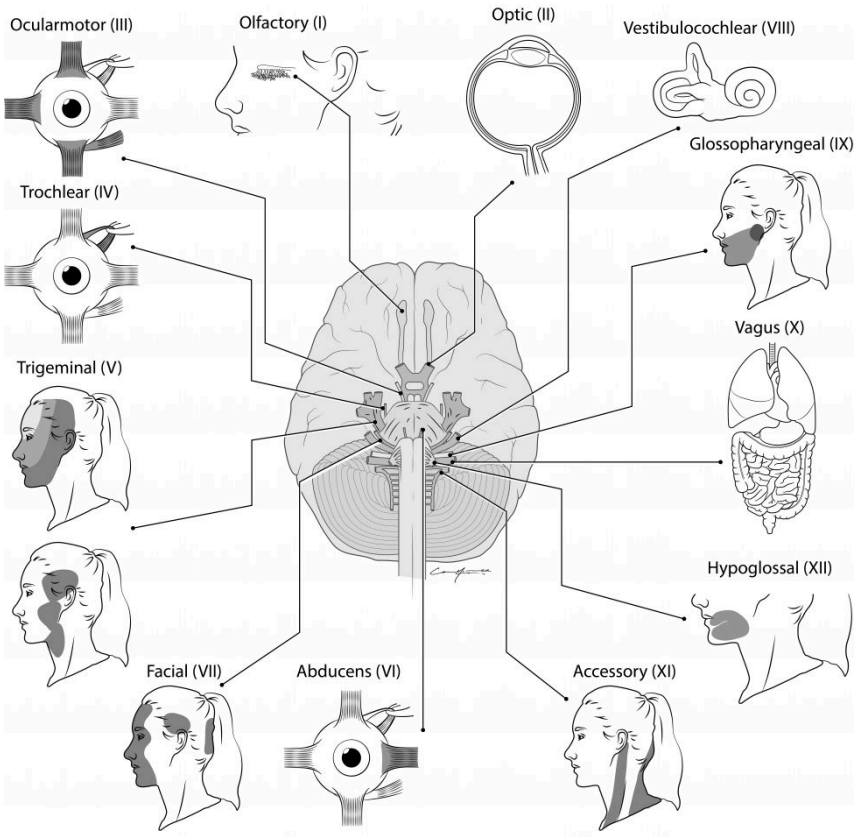


Figure 1a Cranial Nerve Pathways. Figure created by Cameron Miller, licensed CC-by-ND.

Cranial nerve function is commonly assessed as part of a general physical examination of the head, eyes, ears, nose, throat, and neck by physicians. More comprehensive examination of cranial nerve functions is usually done by specialists such as neurologists, ophthalmologists, optometrists, and audiologists.

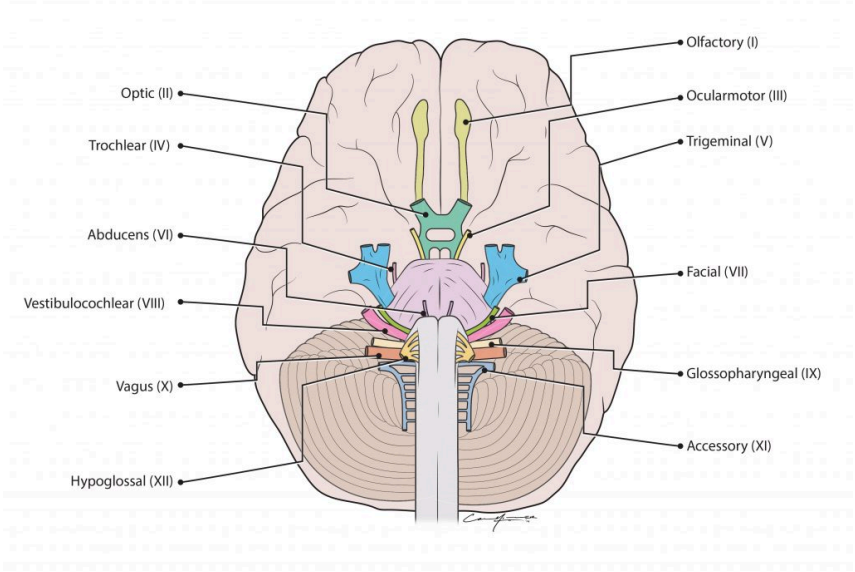


Figure 1b. Ventral aspect of the brain and cranial nerve origins. Figure created by Cameron Miller, licensed CC-by-ND.

Cranial Nerve I. Olfactory Nerve (Sensory)

Olfactory nerve fibers pass from bipolar olfactory neurons in the olfactory epithelium through the cribriform plate of the ethmoid bone and into the olfactory bulb, where they synapse with second-order neurons called Mitral cells. Neurons of the olfactory bulb transmit olfactory information along the olfactory tract to the olfactory cortex of the cerebrum. Damage to the olfactory epithelium, olfactory nerves, olfactory bulbs and tracts, or the olfactory cortex may produce a loss of the ability to smell (anosmia). The most common test for anosmia involves asking a subject to close their eyes and identify a scent.

Usually anosmia is due to damage to the nasal olfactory epithelium from excessive smoking, cocaine use, or inflammation due to infection. Neurologic causes include tumors of the frontal lobe near the olfactory bulbs and tracts and head injuries. As do other special senses (e.g., vision, hearing), the sense of smell diminishes with increasing age.

Clinical notes: Alzheimer's Disease. Neurodegenerative changes in the olfactory bulb and brain regions that involve smell, including olfactory memory, have been associated with the onset of Alzheimer's Disease (AD) dementia. A simple sniff test has been used to identify Alzheimer's versus non-Alzheimer's patients. In the test, blindfolded patients are asked to smell and identify peanut butter with the right or left nostril 10 cm from the nose. People with Alzheimer's have trouble smelling the peanut butter, and, additionally, have a harder time smelling with the left nostril than the right. Because the sense of smell is often the first sense to be noticed in cognitive decline (before memory loss) the sniff test could be an effective tool for early intervention.

Cranial Nerve II. Optic Nerve (Sensory)

The optic nerve arises from cells in the retina of the eye and conveys visual information to the brain. Fibers from the medial half of each retina cross at the optic chiasma and are distributed to the contralateral brain stem, thalamus, and cerebral visual areas along with fibers from the ipsilateral temporal retina (see figure 2). Thus, both eyes are represented in the occipital cortex of each cerebral hemisphere. In addition to providing sensory information regarding vision, the optic nerve provides the sensory component of visual reflexes such as the pupillary light reflex and accommodation of the lens. Examination of the optic nerve involves determination of visual acuity, peripheral vision, and appearance of the optic fundus with an ophthalmoscope.

Visual acuity is checked with Snellen charts, which display letters of progressively smaller size. "Normal" vision is 20/20. This means that the test subject sees the same line of letters at 20 feet that person with normal vision sees at 20 feet. 20/40 vision means that the test subject sees at 20 feet what a person with normal vision sees at 40

feet. Another way of saying this is that a person with 20/40 vision has vision that is only half as good as normal.

Loss of vision in all or part of the visual field is caused by specific lesions in the visual pathway (figure 2). Blindness (represented by dark color) in one half of each visual field is called hemianopia. Lesions of one optic nerve will cause complete vision loss in the ipsilateral eye. Lesions of the optic tract or optic radiations produce the same hemianopia (homonymous hemianopia) for both eyes. A tumor of the pituitary gland may exert pressure on the optic chiasma causing bilateral loss of the temporal fields of vision.

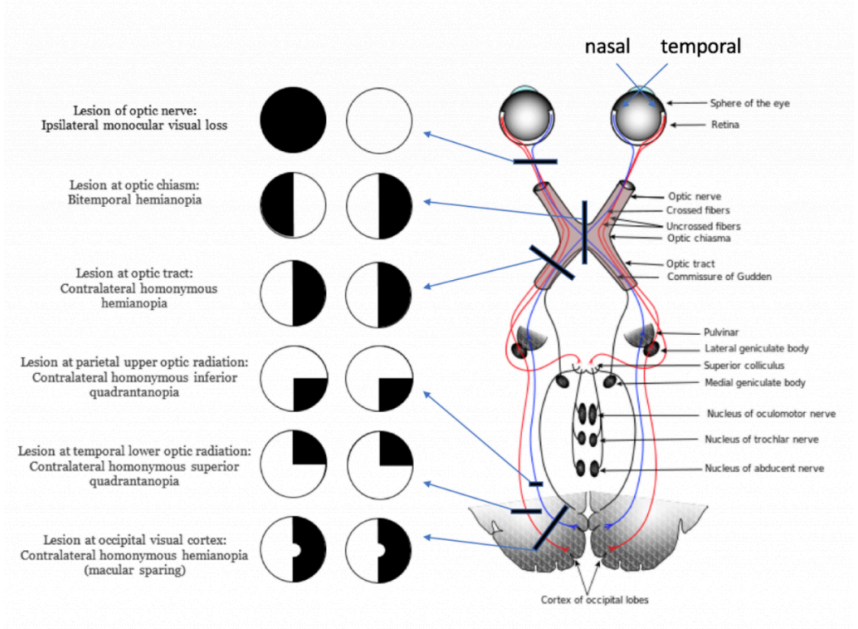


Figure 2. Visual pathways, lesions, and resulting defects in the visual field. Note that in the retina, medial fibers detect the temporal field. Medial fibers decussate at the optic chiasma while lateral fibers run on the ipsilateral side.

Cranial Nerves III: Oculomotor, IV Trochlear, VI Abducens

Six extrinsic muscles move the eyeball within the orbit (figure 3). The inferior, superior, and medial recti muscles and the inferior oblique muscle are controlled by

the **oculomotor nerve**. The lateral rectus is controlled by the **abducens** nerve, and the superior oblique muscle is controlled by the **trochlear** nerve.

The oculomotor nerve also controls the elevator muscle of the upper eyelid and the involuntary internal muscles of the eye that control pupil diameter and lens thickness. Because the oculomotor, trochlear, and abducens nerves control related functions of the eye, they are tested as a unit.

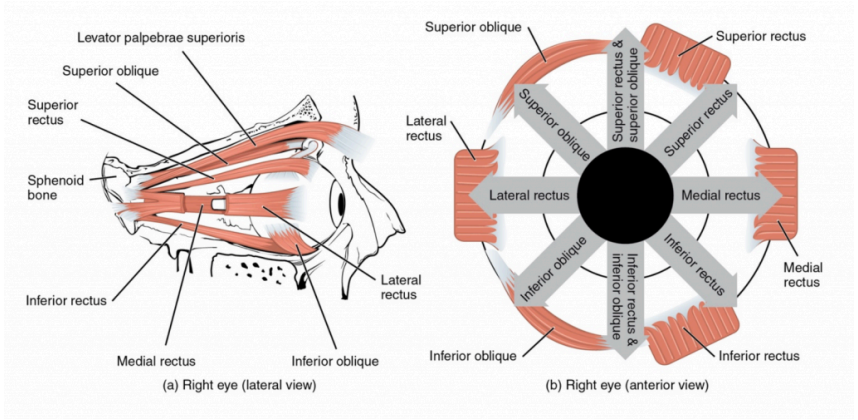


Figure 3. Extrinsic eye muscles, eyeball movement, and cranial nerve controls. Images courtesy of OpenStax A&P and Anatomy and Physiology (Open + Free), licensed **CC Attribution 4.0**

Cranial nerve controls. Courtesy of OpenStax A&P and Anatomy and Physiology (Open + Free), licensed CC Attribution 4.0

Muscle	Effect of Contraction	Innervated by (including cranial nerve number):
Superior rectus	Eye rotates to look up	Oculomotor nerve (III)
Medial rectus	Eye rotates to look medially	Oculomotor (III)
Inferior rectus	Eye rotates to look down	Oculomotor (III)
Lateral rectus	Eye rotates to look laterally	Abducens (VI)
Superior oblique	Medial rotation	Trochlear (IV)
Inferior oblique	Lateral rotation	Oculomotor (III)

Tests of these nerves involve looking at the eye structure, eyelid, and eye movement.

Damage to the oculomotor nerve causes several unusual eye features, including **ptosis**, or drooping of an eyelid. Unequal pupils, called **anisocoria**, may be congenital and have no pathologic significance or may occur as a result of a variety of abnormalities, including syphilis, multiple sclerosis, and sympathetic paralysis. If both pupils are markedly smaller or larger than normal, medication may be the cause. A unilateral dilated pupil often occurs with increased intracranial pressure. The pupil becomes fixed and unresponsive to light on the side of the brain where the pressure has increased.

Extraocular muscles are tested by the **H test** where the subject is asked to follow a pen or finger tracing the shape of an H with their eyes. The ability to rotate the eyes medially, called **convergence**, allows the subject to focus on an object that is close to the face. **Nystagmus**, or involuntary, rapid, rhythmic shaking movement of the eyeball, may occur due to extraocular muscle weakness.

Cranial Nerve V. Trigeminal Nerve (Mixed)

The trigeminal nerve has two roots, one motor and the other sensory, arising from the pons (figure 1b). The motor fibers supply muscles of mastication (chewing), such as the **temporalis and the masseter**. The sensory fibers convey pain, temperature, touch, and pressure information from the eye, face, nasal and oral mucosa, gums, teeth, and anterior two-thirds of the tongue.

Fibers in the sensory root come from the trigeminal ganglion, which receives input from three divisions of sensory nerves: the ophthalmic (I), the maxillary (II), and the mandibular (III). The facial distribution of these divisions is shown in figure 4. Each division should be tested separately and bilaterally (on both sides).

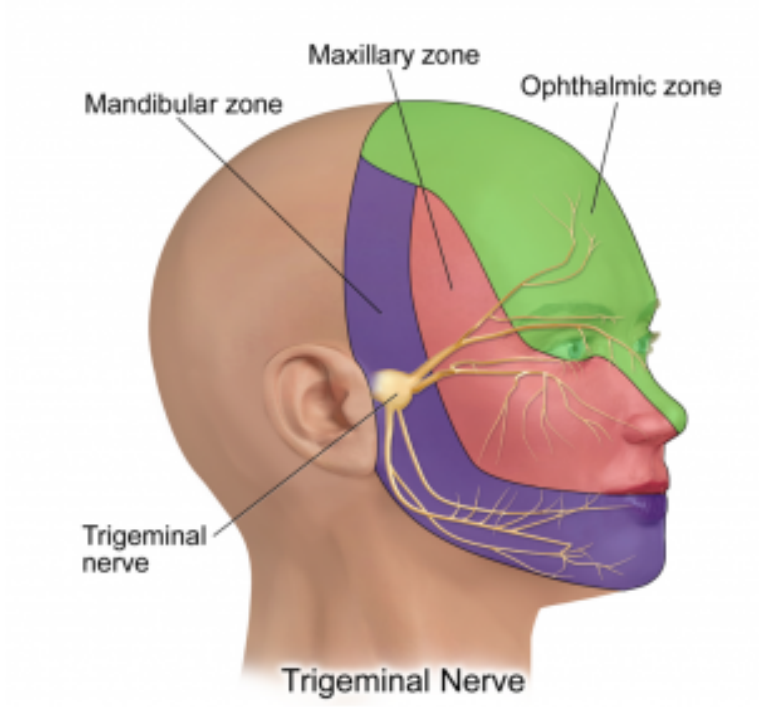


Figure 4. Cutaneous distribution of ophthalmic (green), maxillary (red), and mandibular (purple) divisions of the **trigeminal sensory nerves. Blausen Medical Communications, Inc. CC BY 4.0.**

Injury to the sensory components of the trigeminal nerve causes anesthesia in the area of the affected division and, thus, may be tested for by a simple touch test. Tic douloureux (*trigeminal neuralgia*) is caused by irritation of the trigeminal nerve and is marked by excruciating pain that follows the distribution of the sensory fibers.

Cranial Nerve VII. Facial Nerve (Mixed)

The facial nerve arises from the pons lateral to the abducens nerve (figure 1b) and contains motor and sensory fibers. The motor fibers innervate muscles of facial expression, and parasympathetic fibers stimulate salivary glands. Sensory fibers convey taste information from the anterior two-thirds of the tongue.

One may test for facial nerve function by asking the subject to show teeth and smile,

lift the eyebrows, frown, and close the eyes tightly. All facial movements should be equal bilaterally. Note any asymmetry of facial movements and features, but keep in mind that some persons habitually smile more on one side of the mouth than the other and that every face is somewhat asymmetrical.

Disease of the facial nerve results in peripheral facial paralysis (Bell's palsy) on the side of the lesion. Causes of Bell's palsy include compression of the nerve by a tumor and infections that inflame the nerve and surrounding tissue. The affected individual will be unable to close the eye on that side, wrinkle his or her forehead, or show teeth. Loss of muscle tone on the side of the lesion causes the corner of the mouth to droop.

Cranial Nerve VIII. Vestibulocochlear Nerve (Sensory)

The eighth cranial nerve contains fibers of the vestibular nerve and the cochlear nerve. The vestibular nerve is sensory from receptors in the inner ear that provide information concerning the movement of the body, balance, and body position in relation to gravitational force. The cochlear nerve is sensory from auditory (hearing) receptors in the cochlea of the inner ear.

The inability to hear some or all of the normally audible sounds could be caused by one or more of several disorders such as blockage of the outer ear canal (with wax, fluid, etc.): damage to the eardrum or ear ossicles: blockage of the internal auditory meatus and inflammation of the middle ear (common in upper respiratory infections); damage to the inner ear, auditory (hair cell) receptors, and auditory (cochlear) nerve; or damage to auditory pathways in the brain.

A useful test of hearing requires a 256-Hz tuning fork vibrating on the mastoid process (to test bone conduction) versus placing the fork directly in front of the ear (testing air conduction). A shortening of hearing time with air conduction coupled with preservation of hearing time with bone conduction suggests interference with sound transmission in the outer ear or the middle ear. If both the hearing time for bone conduction and the hearing time for air conduction are reduced, the hearing loss most likely involves the inner ear receptor hair cells or the auditory nerve (or both).

Cranial Nerve IX. Glossopharyngeal Nerve (Mixed), Cranial Nerve X. Vagus Nerve (Mixed)

The glossopharyngeal nerve supplies motor fibers to the parotid salivary gland and muscles in the pharynx (throat), larynx (voice box), and soft palate. Sensory fibers convey taste information from the posterior one-third of the tongue and information pertaining to blood pressure and blood chemistry from the carotid artery.

The vagus nerve supplies motor fibers to constrictor muscles of the pharynx, intrinsic muscles of the larynx, and involuntary muscles of the bronchi, heart, esophagus, stomach, small intestine, and part of the large intestine. Secretory motor fibers of the vagus supply the pancreas and secretory glands of most of the alimentary canal. The vagus is sensory from the laryngeal mucosa, heart, lungs, esophagus, stomach, small intestine, and part of the large intestine. In addition, vagal sensory fibers convey taste from the throat and epiglottis and blood pressure and chemistry information from the aorta. The ninth and tenth cranial nerves are tested together because their functions overlap.

A common test to determine IX and X function is to ask a subject to open his or her mouth and say "ahh." The position of the soft palate and uvula at rest and with phonation is noted. Normally, the uvula and palate rise in the midline with phonation. Paralysis of cranial nerves IX or X on one side of the brain will cause the palate and uvula to deviate to the un-paralyzed side during phonation (figure 5).

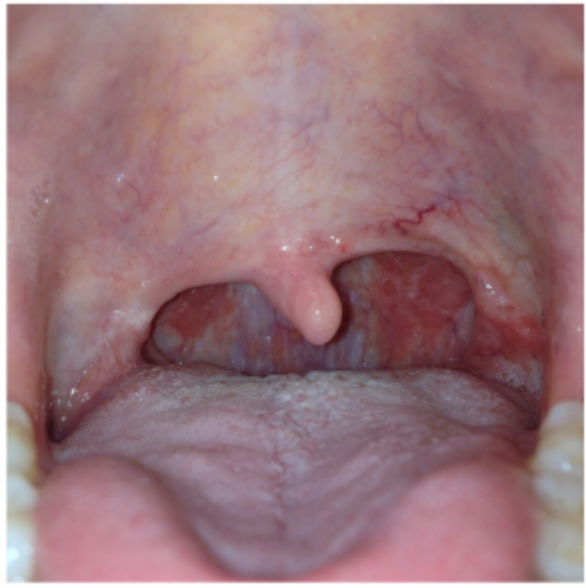


Figure 5. Deviation of the uvula to the left side due to nerve damage. CC Attribution 3.0

Additionally, the absence of the gag reflex may result from a lesion of either the glossopharyngeal (sensory component) or vagus nerve (motor component) on the same side as the loss. If an abnormality is suspected, taste is tested on each side of the posterior one-third of the tongue.

Cranial Nerve XI. Spinal Accessory Nerve (Motor)

The eleventh cranial nerve supplies some motor fibers to the muscles of the larynx and pharynx via the pharyngeal plexus (C.N. IX-X-XI), but its principal distribution is motor to the **sternocleidomastoid** and **trapezius** muscles (Fig. 6). The sternocleidomastoid turns the head to the opposite side, and the trapezius muscle elevates the shoulder on the same side. Function of cranial nerve XI is easily determined by checking for bilateral muscle resistance.

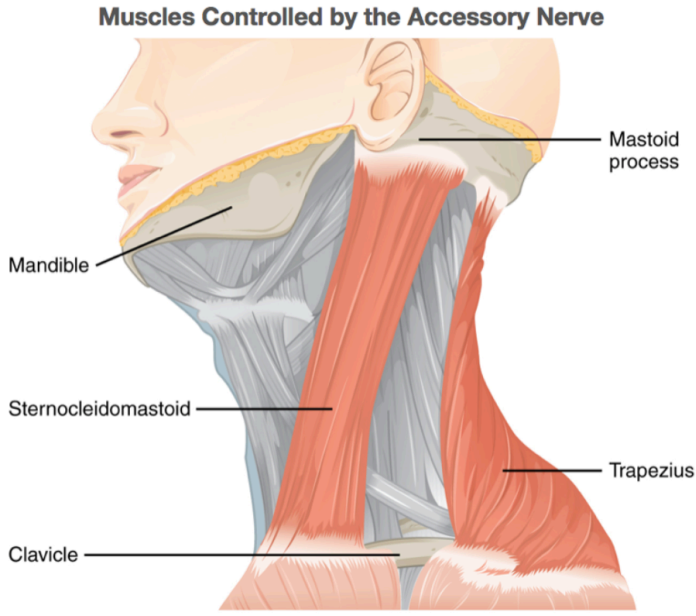


Figure 6. Muscles controlled by the accessory nerve. Image courtesy of OpenStax A&P **CC Attribution 4.0.**

Cranial Nerve XII. Hypoglossal Nerve (Motor)

The hypoglossal nerve supplies motor fibers to muscles of the tongue. Muscles in the right half of the tongue are supplied by the right hypoglossal nerve, and muscles in the left half are supplied by the left hypoglossal nerve.

To determine hypoglossal nerve function, one would assess the subject's speech by asking the subject to read aloud. Movement of the tongue is important in forming the vowel and consonant sounds of speech.

Additionally, a practitioner may ask the subject to protrude his or her tongue in the midline to check for deviation of the tongue tip to the right or left and for atrophy of muscle in the right or left half of the tongue. Normally the tip of the protruded tongue will not deviate from the midline and the tongue will appear symmetrical. A lesion of the hypoglossal nerve will cause the protruded tongue to deviate toward the affected side, and the appearance of the tongue will be asymmetrical owing to

atrophy of muscle on the affected side. Often, fasciculations (brief, spontaneous contractions) of the tongue muscle occur on the affected side, indicative of motor neuron damage.

Laboratory Methods

Exercises

This laboratory focuses on the standard clinical techniques for the assessment of the cranial nerves.

IMPORTANT: Have your lab report available during the assessment to keep track of test results.

Olfactory nerve (CN I)

The olfactory nerve is a pure sensory cranial nerve that transmits olfactory information to the brain.

Materials: Vials containing the following: vanilla extract, ground coffee, ammonia, and a small metric ruler

Procedure:

- Have your lab partner close their eyes and block one nostril by occluding it with their finger.
- Place the substance vial approximately 10 centimeters below the unobstructed nostril. Ask them to inhale normally and identify the scent.
- **Recap each vial before opening the next scent.**

- Record their answer on the lab worksheet.
- Repeat procedure with all vials, alternating the nostril tested until both nostrils have been tested using all scents.

Optic nerve (CN II)

The optic nerve is a sensory cranial nerve, which carries visual information to the brain.

Materials: Snellen eye chart and a pen (or pencil)

Procedure: Examination of the optic nerve involves a determination of visual acuity and peripheral vision.

Visual acuity: Acuity is the sharpness of a visual image. Use the Snellen eye chart located on the lab wall to perform this test. Record your observations on the lab worksheet.

- Have the subject stand 20 feet (6.1 m) away from and facing the chart.
- Have the subject cover one eye and read the letters on the chart you point to for them, starting with the top line and working to the bottom.
- Note the lowest row of letters that can be read accurately.
- Record the number of that row in the worksheet (use the bottom number in the ratio printed to the left of the row of letters). This number is the farthest distance (in feet) that a person with normal acuity can see the letters in that row.

Example

- If a subject has 20-20 vision, that subject can see at 20 feet what a person with normal vision can see at 20 feet. If the subject's vision is

20-40, the subject sees at 20 feet what a normal subject can see at 40 feet.

- Test both eyes and record the numbers; if the subject wears glasses, you can try the chart with and without glasses (make sure to note in your worksheet whether the subject wore glasses or not during the test).

Peripheral vision

This test assesses peripheral vision:

- Have your lab partner sit directly in front of you at the same eye level height and at a distance of about 2 feet.
- Have them cover their right eye and look directly at you with their left eye. Make sure they continue to stare directly ahead during this procedure.
- To perform stationary testing:
    - The examiner closes his or her eye directly opposite the patient's closed eye and holds up a certain number of fingers peripherally, equidistant between their self and the patient.
    - The patient is asked to correctly identify the number of fingers.
    - All 4 quadrants (upper and lower, temporal and nasal) should be tested (see figure below).
    - Repeat with the other eye.
    - Record whether the patient correctly identified the number in each quadrant on the lab worksheet.

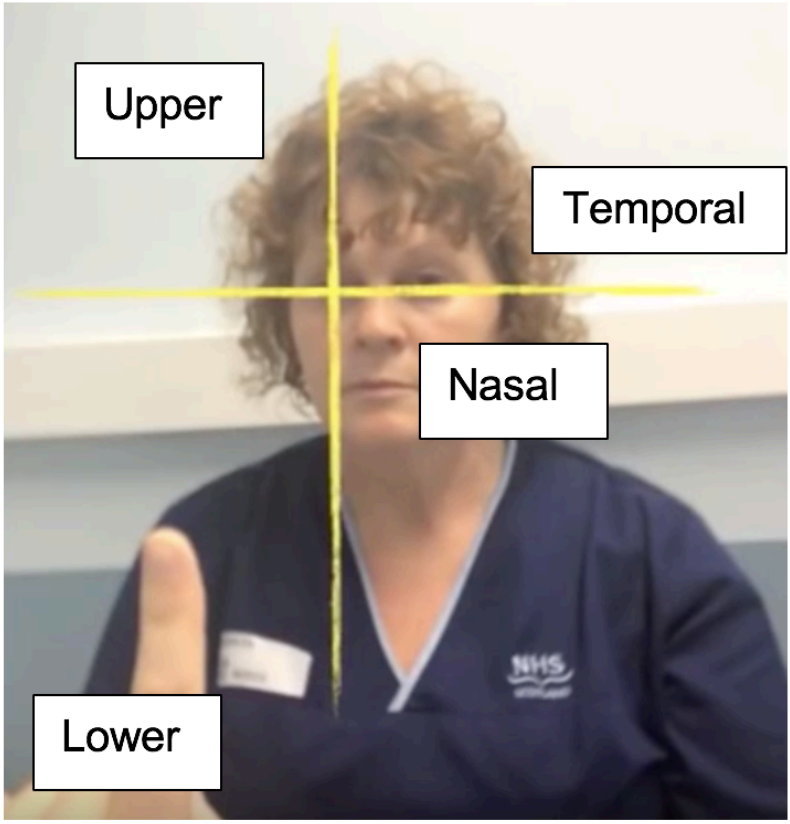


Figure 7. Quadrants for the peripheral vision test.

Oculomotor (CN III)

Six extrinsic eye muscles are responsible for eyeball movements. Three cranial nerves control these muscles. Four of these six muscles are controlled by the oculomotor nerve, which are the superior, inferior and medial recti and the inferior oblique muscles.

Materials: Each pair of students should have: Penlight, pen or pencil, metric ruler

Procedure to test for motor functions:

- Stand directly in front of the student to be tested.
- Ask them to stare directly ahead.
- Have the student follow with their eyes as you trace the letter H in space.
- Look for smooth coordinated movement with both eyes.
- Record your observations in the lab worksheet.

The oculomotor nerve controls the *levator palpebrae superioris* muscle that raises the upper eyelid.

- Ask your lab partner to stare directly ahead.
- Check the position of each eyelid. Are they symmetrical?
- Normally about one third of the iris of the eye should be covered by the eyelid.
- Record your observations on the worksheet.

The oculomotor nerve also carries **parasympathetic fibers** to control the sphincter pupillae. Activation of this smooth muscle causes pupil constriction. The ciliary muscle also receives parasympathetic fibers via the oculomotor nerve. This muscle is responsible for lens accommodation for close vision.

Procedure to test for pupillary constriction:

- As your lab partner is looking directly ahead look to see if both pupils are the same size.
- Are they round or oval?
- Are they facing forward?
- To maximize results from the pupillary constriction test, you may conduct the test in a dark lab, as long as you can still see the pupils of the subject's eyes.
- Have your lab partner focus on an object in the distance straight ahead.
- Hold the penlight about 20 centimeters to the side of the Right eye and direct the light toward the pupil.
- Did the pupil constrict?
- Repeat shining the light on the Right eye and check to see if this results in the

same pupillary response in the Left eye (consensual response).
- Repeat this procedure by shining the light onto the Left eye.
- Record your observations.

Procedure to test for accommodation:

- Have your lab partner stare straight ahead at a distant object.
- Take a pencil and place it about **1 meter** in front of your lab partner's gaze.
- Have your lab partner follow the pencil as you move the pencil to about **3 centimeters** in front of their nose.
- Look for convergence of the eyes and pupillary constriction as the subject follows the path of the moving pencil.
- Record your observations.

Trochlear nerve (CN IV)

The trochlear nerve is a pure motor nerve, which supplies one of the six extrinsic eye muscles, the superior oblique. The action of this muscle is to bring the downward and inward.

Procedure to test for motor function:

- Have your lab partner look down at the tip of their nose.
- Observe the movement in both eyes.
- Is the movement symmetrical and smooth?
- Record your observations.

Trigeminal nerve (CN V)

The trigeminal has both motor and sensory components. The motor fibers control the muscles of mastication and the sensory fibers convey the sensations of pain, touch, temperature and pressure from the face, eyes, gums, oral and nasal mucosa, teeth and the anterior two thirds of the tongue. There are three major divisions of the trigeminal nerve: the ophthalmic, maxillary, and mandibular.

Materials: Each student should have: cotton ball (1 per student), tongue depressor (please do not throw away the tongue depressor: each student should keep their own tongue depressor for this and other portions of the clinical exam).

Procedure to test motor functions of the trigeminal nerve:

- Ask your lab partner to clench their teeth several times.
- While your lab partner is clenching their teeth, **palpate** the temporalis muscle on each side of the head.
- Is the contraction of equal strength on each side?
- Test the strength of masseter muscles using the same technique.
- The masseter can be palpated just above and to the front of the angle of the lower jaw.
- Contractions should be symmetrical and equal.
- Ask your lab partner to bite down on a tongue depressor on the right side.
- Try and pull the tongue depressor out.
- Repeat this procedure on the opposite side
- Record your observations.

Procedure to test sensory functions of trigeminal nerve:

- Three divisions of the trigeminal nerve will be tested for sensory function.
- With your lab partner's eyes closed take a cotton ball and touch the skin lightly in several different locations.
- To test the **ophthalmic division (V1)** use described procedures on the forehead just above the **glabella,** slightly to the left and slightly to the right.
- The **maxillary division (V2)** is tested on the skin of the buccal region on the left side and then on the right side.
- The **mandibular division (V3)** is tested on the mental region slightly to the left side then slightly to the right side. **See figures below.**
- Ask them to indicate if they feel the touch.
- Record your observations.

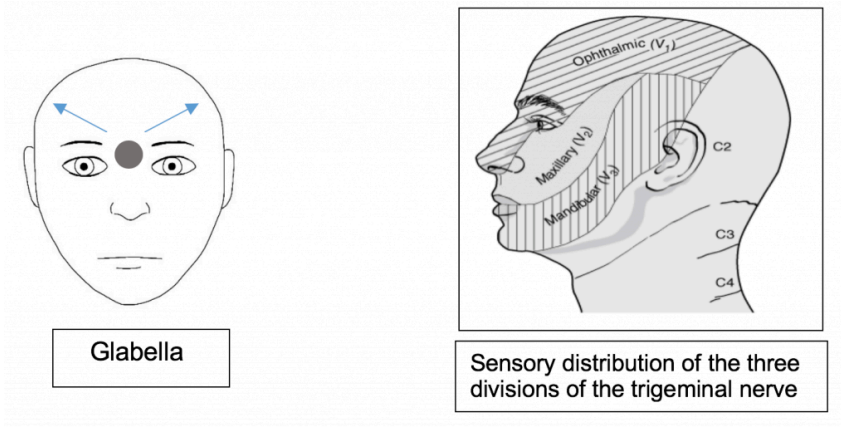


Figure 8. Location of the glabella and sensory distribution of the trigeminal nerve.

Abducens nerve (CN VI)

The abducens nerve is a motor nerve, which supplies one of the six extrinsic eye muscles, the lateral rectus. The action of this muscle is to abduct the eye (move it laterally)

Procedure to test for motor function:

- Have your lab partner stare straight ahead
- Then move the eyes to **the right** and **then to the left.**
- Observe the movement in both eyes.
- Is the movement symmetrical and smooth?
- Record your observations.

Facial nerve (CN VII)

The facial nerve is a mixed cranial nerve that also has parasympathetic fibers associated with it. The motor component controls the muscles of facial expression. The sensory portion conveys taste information from the taste buds located on the anterior two-thirds of the tongue. **Parasympathetic** fibers innervate the

submandibular and sublingual salivary glands, the lacrimal (tear) glands of the eyes, and nasal and palatine glands.

Procedure to test motor component of the facial nerve:

- Ask you lab partner to:
  - smile
  - lift their eyebrows
  - frown
  - close their eyes tightly
  - pucker their lips
  - wrinkle the forehead

Look for symmetry of the muscles on both sides of the face.

Materials: Each pair of students should have: natural sugar solution, salt solution, 8 disposable pipets, 2 10ml beakers per student

Procedure to test for sensory function of the facial nerve:

Notes: During this procedure, never touch the pipet to the tongue.

Pour 5ml of each solution into a beaker and use a new pipette each time you take solution from the beaker.

- Ask your lab partner to **close their eyes and protrude their tongue**.
  - Ask your partner to raise their hand when they taste a solution.
  - Ask your partner what solution they detected.
- Place a few drops of the **sugar solution** on the anterior half of the tongue on

the **right side**.

- Place a few drops of the **sugar solution** on the anterior half of the tongue on the **left side.**
- Place a few drops of the **salt solution** on the anterior half of the tongue on the **right side.**
- Place a few drops of the **salt solution** on the anterior half of the tongue on the **left side**
- Record your observations.

Vestibulocochlear nerve (CN VIII)

The vestibulocochlear nerve is a sensory nerve that conveys sound information and balance (equilibrium).

Materials: tuning fork

1. Rinne Test.

This test is used to identify impairment in the conduction of sound though the external and middle ear, to the sensory areas of the inner ear. It is done by comparing air conduction with bone conduction.

- Strike a tuning fork against the palm of your hand (never strike the tuning fork against a hard surface)
- Place its handle on the subject's mastoid process.
- When the subject no longer hears the sound, move the prongs (they should still be vibrating) to the opening of the external auditory meatus (be careful not to touch it). *Normally, the hum will be heard again; if not, conduction impairment is suspected.*
- Record your results in the worksheet.
- Repeat on the other side of the head.

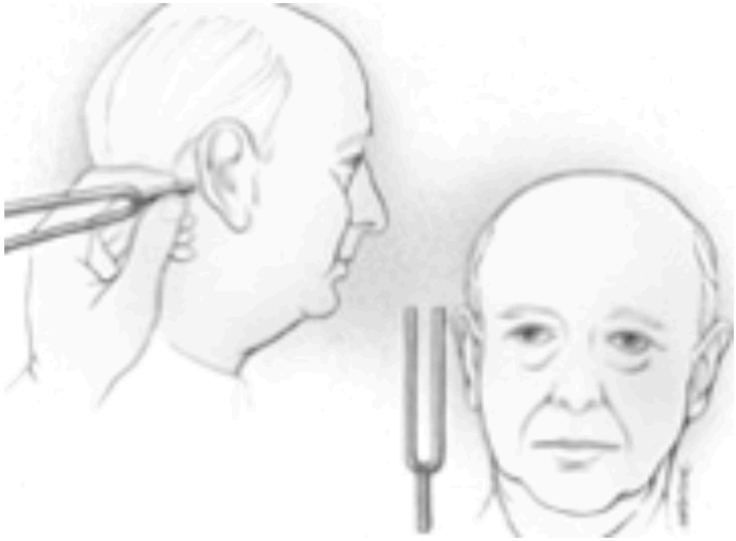


Figure 9. Placement of the tuning fork behind next to the ear (not touching the subject) and then with the base of the tuning fork touching the subject on the bone behind the ear.

2. Weber Test.

This is another test used to identify defects in conduction. The Weber Test is only useful if there is an asymmetrical hearing loss. If hearing is symmetrical, the patient perceives the sound in the middle of their head.

- Strike a tuning fork against the palm of your hand and place the handle against the middle of the subject's forehead.
- Ask the subject on which side, if any, the sound seems louder.
- Record your results.

Figure 10. Placement of the base of the tuning fork touching the middle of the subject's forehead for the Weber Test.

3. Romberg Test.

The Romberg test evaluates a subject's ability to maintain balance and equilibrium, using proprioceptors and the vestibular apparatus (utricle and saccule) without the aid of vision. Impairment of the dorsal white column of the spinal cord (transmits information from proprioceptors to the brain), the vestibular apparatus, the basal ganglia or the cerebellum can be detected during this test. **Note:** If equilibrium impairment exists, the subject may topple over. Have at least one person standing by to stabilize the subject if this happens.

Procedure:

- Have the subject stand near the wall with the tape on it.
- Have the student stand so you can see at least one set of lines on each side the subject's body, **parallel** to it.
- **Have the subject stand in place, feet together, with their eyes open, staring straight ahead for 30 seconds. Note any swaying movements,**

using the lines on the board as a reference.

- **Repeat the test, this time with the subject's eyes closed. Again, note the degree of side-to-side movement.**
- Repeat the test again (**steps in bold above**) with the subject standing **perpendicular** to the wall (the left shoulder is near the wall, but not touching it)

4. Auditory Frequency Test.

Determine the subject's range of hearing by listening to a spectrum of sounds from YouTube. Use the headphones, but please make sure the volume is turned to 30 (right side lower task bar), or higher frequency sounds will be unpleasant.

Use the spectrum to determine the low and high frequency values for your hearing.

Put on the headphones. Open the "Week5_20Hz to 20 kHz" file from the P-drive. Click pause to record when you start hearing and when you stop hearing the sounds.

Glossopharyngeal nerve (CN IX) and Vagus nerve (CN X)

The glossopharyngeal nerve is a mixed nerve that also supplies **parasympathetic** innervation to the parotid salivary glands in the head. The motor fibers of the glossopharyngeal supply skeletal muscles of the pharynx, larynx, and soft palate. Sensory fibers convey taste information from taste buds on the posterior one third of the tongue. Sensory information concerning blood pressure and blood chemistry is also found in this cranial nerve.

The vagus is a mixed nerve with **parasympathetic** fibers. The parasympathetic fibers innervate structures in the neck, thorax and into the abdomen. The motor fibers of the vagus supply skeletal muscles of the pharynx and larynx. Some taste buds are found around the epiglottis. Sensory information from these taste buds is conveyed by the vagus nerve.

Many functions of the glossopharyngeal and vagus nerves overlap therefore typically; these two nerves are tested together.

Materials: Tongue depressor, penlight, soy sauce solution, 8 disposable pipets

Procedure:

- Ask your lab partner to open their mouth and say "ahh".
- Hold down the tongue with the depressor and using the pen light look at the position of the soft palate and uvula before and during the test.
- During phonation (when they are saying "ahh") the uvula should rise in the midline position.
- Check vocalization by asking them to read a sentence from the lab instructions. Is there any unexplained hoarseness?
- Ask your lab partner to swallow. Do they have any difficulties?
- Record your observations

Glossopharyngeal nerve (CN IX) and Vagus nerve (CN X)

Notes on procedure to test for sensory function of the CN IX & CN X nerve:

During this procedure, never touch the pipet to the tongue

Use a new pipette each time you take solution from the beaker

Pour 5ml soy sauce solution into a beaker.

- Ask your lab partner to **close their eyes and protrude their tongue**.
- Place a few drops of the **soy sauce** on the **posterior** half of the tongue on the **right side**.
- Place a few drops of the **soy sauce** on the **posterior** half of the tongue on the **left side.**
- Have your lab partner raise their hand when they taste the solution.
- Have them report the taste of the solution that was placed on their tongue.

- Record your observations.

Accessory nerve (CN XI)

The accessory nerve has motor function, and it transmits motor fibers to the sternocleidomastoid and trapezius muscles.

Procedure:

- Have your lab partner stare straight ahead.
- Place your hand on their right cheek.
- Have them turn their head to the right against the resistance of your hand.
- Repeat this procedure on the left side.
- For each side, assess the strength of the muscle contraction using a scale of 1 (very weak) to 4 (very strong).
- Observe whether there are any differences in the strength of the muscle contraction between the two sides.
- Record your observations.
- Have your lab partner sit up straight on the lab stool.
- Place your hands on their shoulders.
- Ask them to shrug their shoulders against resistance.
- Assess the strength of the contraction using the scale mentioned above.
- Compare the strength of the contraction between the two sides
- Record your observations.

Hypoglossal nerve (CN XII)

The hypoglossal nerve is a motor nerve to the intrinsic muscles of the tongue.

Procedure:

- Ask your lab partner to protrude their tongue in the midline.
- Look for any deviations to the right or left.
- The tongue should not deviate from the midline.
- Record your observations.

Online Resources:

- UC San Diego Practical Guide to Clinical Medicine, Cranial Nerves
- The Cranial Nerve Practice Exam, OpenStax
- Cranial Nerves Lab [download doc]
- OpenStax College. (2013). *Anatomy & physiology*. Houston, TX: OpenStax CNX. Retrieved from http://cnx.org/content/col11496/latest/

PATELLAR AND ACHILLES REFLEXES

Karri Haen Whitmer

A reflex is an involuntary (automatic) response to stimulus that quickly returns the body to homeostasis. There are several kinds of reflexes. Examples are shivering in response to low core body temperature; or withdrawing your hand from a hot stove when temperature and pain receptors in your hand register the stimulus.

A reflex arc refers to the neural pathway that a nerve impulse follows. The reflex arc typically consists of five components:

1. A receptor, and independent sensory cell, or an ending of a sensory neuron, reacts to a stimulus (e.g., a stretch receptor).
2. The sensory, or **afferent**, neuron sends a nerve impulse through an afferent pathway to the central nervous system.
3. An **integration center** consists of one or more synapses in the CNS (typically the spinal cord) where the incoming information and outgoing response are integrated.
4. A motor, or **efferent**, neuron sends a nerve impulse along an efferent pathway from the integration center to an effector cell.
5. The effector cell responds to efferent impulses (for example, by contracting, if the effector is a muscle fiber).

Because integrating center processing may occur at the level of the spinal cord rather than requiring impulses to travel to the brain, reflex responses have a relatively short **path length** and, thus, a quick **reaction time** compared to **voluntary** or conscious body movements.

Reflexes require a minimum of two neurons, a sensory neuron (input) and a motor neuron (output) (see Figure 1). The sensory neuron detects stimuli and sends a signal towards the CNS. The sensory neuron synapses with a motor neuron, which innervates an effector tissue, such as skeletal muscle in order to pull away from painful stimuli. This type of reflex is a "withdrawal" reflex and is **monosynaptic**, meaning only one synapse has to be crossed between the sensory neuron and the motor neuron. This is the simplest reflex arc, and the integrating center is in the spinal cord. **Polysynaptic** reflexes are more complex, but also more common. They involve interneurons, found in the CNS, which further process stimulus and output information. Beyond simple reflexes with integrating centers in the spinal cord, more complex reflexes have integration centers in the brainstem or even in the cerebrum.

A **stretch reflex** is a type of muscle reflex, which protects muscle against increases in length that can tear and damage muscle fibers. The primary purpose of the patellar reflex – the stretch reflex of the *quadriceps femoris* muscle – is to prevent excessive stretching of the quadriceps. The patellar reflex is illustrated in Figure 1.

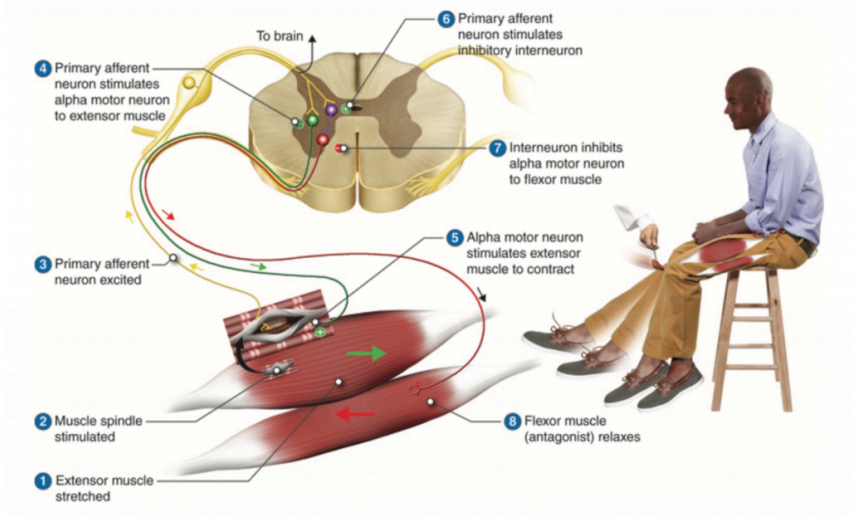


Figure 1. The monosynaptic patellar reflex. A tap to the patellar tendon stretches the quadriceps muscle (1) resulting in activation of the muscle spindle (2). The afferent neuron of the muscle spindle, detecting stretch, sends a signal to the spinal cord (3) and synapses directly with a motor neuron (4) that causes the quadriceps muscle to contract (5). In parallel, an inhibitory message is sent via an interneuron to the hamstrings (6, 7), resulting in hamstrings relaxation (8). The reflex at the hamstrings is polysynaptic. This work by Cenveo is licensed under Creative Commons Attribution 3.0 United States https://creativecommons.org/licenses/by/3.0/us/ .

The **patellar tendon** attaches the quadriceps muscle to the tibial tuberosity of the lower leg. The quadriceps is an extensor muscle: when it contracts it extends the angle of the knee joint by raising the lower leg from a bent position. Tapping the patellar tendon pulls and stretches the quadriceps muscle and causes the sensory receptor of the muscle, called a **muscle spindle fiber**, to send a signal along the afferent neuron to the spinal cord (Figure 2). This causes the efferent neuron to return a signal to the quadriceps muscle to contract and lift the lower leg. This action resists the initial stretch and is a classic example of negative feedback.

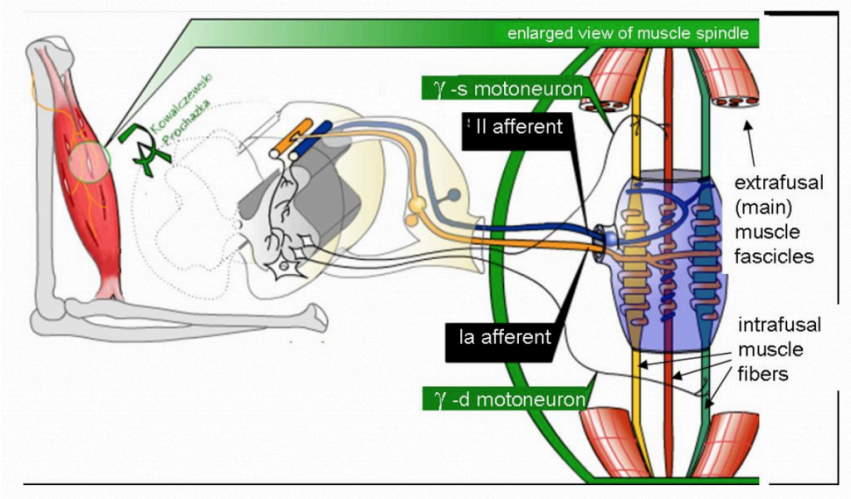


Figure 2. A muscle spindle. Relative motor and sensory innervation shown for the intrafusal fibers inside the spindle. Note the relative placement of extrafusal (contractile) fibers around the spindle. This work is licensed under Creative Commons Attribution US 1.0. https://creativecommons.org/licenses/by/1.0/

To better consider the events that result in the "sensation" of muscle stretch, we must first define muscle fiber types. Muscle spindles, sensory structures which contain **intrafusal muscle fibers**, are innervated by sensory neurons and are arranged in parallel to normal (contractile), **extrafusal**, muscle fibers. Intrafusal fibers are not contractile; instead, they respond to tension by depolarizing a sensory neuron. The sensory neuron synapses with a motor neuron in the spinal cord that innervates contractile extrafusal fibers. The contraction of the extrafusal fibers, that is, contraction of the belly of the muscle, releases tension on the intrafusal fibers, decreasing stimulation to neuron.

In the case of the monosynaptic knee-jerk reflex, hitting the patellar tendon with a mallet stretches the intrafusal fibers of the spindles in the quadriceps muscle, leading to contraction. In parallel, an inhibitory impulse is sent from the spinal cord to cause relaxation of the hamstring muscles, via a polysynaptic pathway.

It is important to note that, even with the simplest of reflexes, there are multiple inhibitory and stimulatory influences that can affect the excitability of the motor neuron. These can amplify or suppress a reflex response and may somewhat vary

from subject to subject. Lesions that damage the sensory or motor fibers, or damage to the spinal cord, generally diminish a reflex unless the spinal cord has been completely transected. In the latter case, spinal cord damage or damage to "upper motor neurons," motor neurons in the brain, may eventually lead to overly reactive reflexes (hyperreflexia).

Additionally, neural activity at other sites in the body may influence a reflex arc. **Facilitation (reinforcement)** may enhance the relative strength (relative amplitude) and/or speed (reaction time, in milliseconds) of a reflex response due to maximal isometric contraction of muscles in a remote part of the body – for example, by clenching the jaw or locking the fingers of the two hands and pulling (the **Jendrassik maneuver**). The Jendrassik maneuver (JM) is a special method for reinforcement that is applied in the clinical setting when it is difficult to initiate a reflex in a patient. The JM likely amplifies reflexes by decreasing inhibitory signals to the alpha motor neurons that are responsible for muscle contraction at the neuromuscular junction (NMJ) (Passmore & Bruno 2012). *Sometimes, though, outside of the clinical setting, experimental outcomes with the JM maneuver can result in no effect or inhibition of a reflex.* Different results when implementing the JM may be due to differences in the physiology of healthy experimental subjects versus patients (in the clinical setting) who are being assessed for nerve problems.

Reflex testing is of clinical value. Testing of **the patellar response** indicates:

- The relative health of the muscle spindle, afferent (sensory) and efferent (motor) neurons, neuromuscular junctions, and the extrafusal (contractile) muscle fibers.
- An appropriate balance of excitatory and inhibitory regulation from the central nervous system.
- The integrity of the L2-L4 vertebral segments of the spinal cord.

Tests for simple muscle reflexes, such as the patellar reflex, are basic to any physical exam when motor nerve or spinal damage is suspected. The tests help to locate neural damage: motor nerves synapsing in the spinal cord above the damage site aren't affected, but nerves that originate at or below the injury will most often produce abnormal reflexes. To determine this, doctors may also test stretch reflexes in the triceps muscle and the **Achilles tendon** (ankle-jerk reflex) and compare results**.**

The **Achilles reflex** is a monosynaptic stretch reflex similar to the patellar reflex. In the Achilles reflex, the hammer taps the Achilles tendon while the foot is dorsiflexed, and the foot, in response, should jerk toward the plantar surface. The Achilles reflex originates in the S1 and S2 nerve roots. When comparing reflexes from different sites of the body, the locations of the corresponding nerve roots along the spinal cord should be considered in order to determine possible sites of injuries and differences in reflex path lengths.

This Week's Lab Technique: Surface Electromyography

In this lab, students will record **electromyograms (EMGs)** for the fibers in a muscle, and use them to determine the time between the stretch of a tendon and the arrival of a motor impulse at the muscle. Surface electromyography (EMG) measures the electrical activity in a muscle by placing **recording electrodes** on the skin over the muscle. To be more precise, an EMG measures fluctuations in the electrical activity of muscles due to muscle cell action potentials.

The EMG produces an electromyogram, which records both the relative amplitude (relative strength) and timing of muscle contractions. The EMG displays the electrical potentials generated by the muscle cells on a computer screen as a series of peaks and troughs defining the EMG wave (Fig. 3, below).

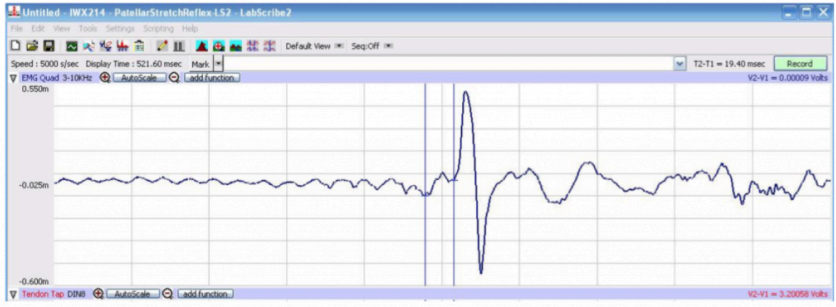


Figure 3. Normal EMG recording for a stretch reflex in LabScribe.

In this lab, two monosynaptic reflexes in a human subject will be studied: the Achilles tendon reflex, and the patellar tendon (knee-jerk) reflex. Mean **reflex reaction**

times, the observed time for the reflex response measured in seconds, and **reflex conduction velocities**, the velocity of transmission in the nerve measured in meters/second, for each reflex arc will be determined and compared. The effect of pre-existing tension in the effector muscle, or motor activity in other muscle groups, upon reflex responses will be measured. The coordination of motor activity in antagonistic muscles may also be studied.

Some measurements we will make in the lab experiments:

1. Response time, path length, and conduction velocity of the patellar and Achilles reflexes.
2. Voluntary vs reflex muscle movement reaction times.
3. Reflex amplitude (strength) and speed with and without reflex reinforcement.

Can you think of some hypotheses for the experiments in this week's laboratory?

Stretch Reflexes Laboratory Methods

Lab Activity Highlights

Stretch reflexes are protective reflexes that ensue to avoid damage due to over-stretching a muscle.

Stretch reflexes occur in response to the activation of special sensory receptors in the muscle called "muscle spindles" or "stretch receptors."

In this lab, students will determine the response time, conduction velocity (speed), and amplitude (strength) of two stretch reflexes: the Achilles reflex at the ankle and the patellar (knee-jerk) reflex.

The velocity of a reflex informs us about the health of the receptors,

neurons, and muscles involved in a reflex and can help to diagnose neuromuscular damage or disease.

Equipment Required

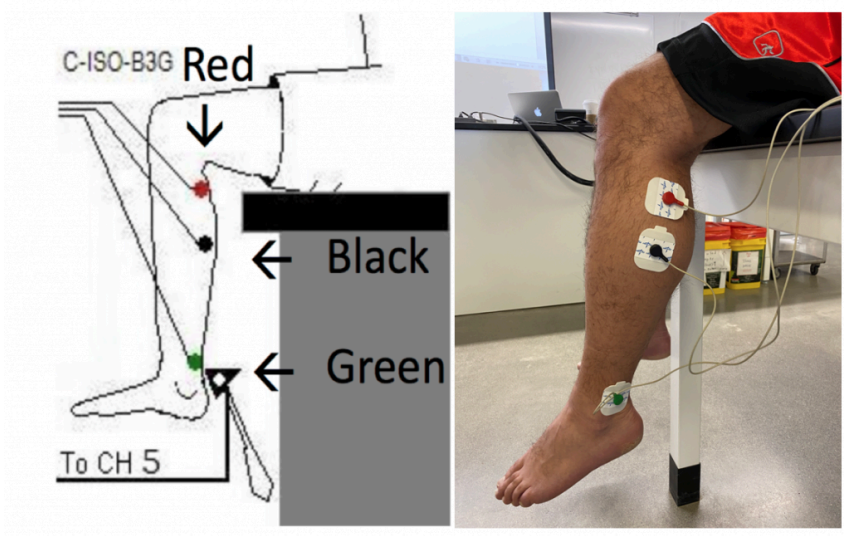
- IXTA data acquisition unit,
- iWire-B3G ECG cable and electrode lead wires,
- alcohol swabs,
- disposable EMG electrodes,
- PRH 200 reflex hammer with BNC connector

Experimental Set-Up: Start the Software

- Turn on the iWorx hardware with the switch on the back of the unit.
- Double click the **Week6 StretchReflex** settings file from the p-drive.

EMG Cable and Reflex Hammer Setup

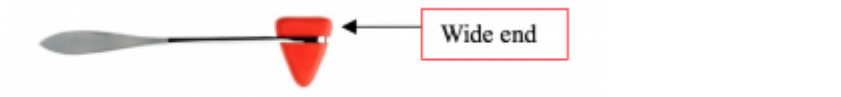
- Use an alcohol swab to clean and abrade three regions on the lower portion of the left leg for electrode attachment. One area is posterolateral **near the knee (see Methods Figure 1)**, the second is posterolateral **on the calf muscles**, and the third area is on **the lateral side of the ankle** that functions as the ground. Let the areas dry.
- Remove the plastic disk from a disposable electrode and apply it to one of the abraded areas. Repeat for the other two areas.
- The **RED** (+1) lead wire is attached to the electrode laterally, **near the back of the knee**. Flex your calf muscle to make sure the electrode is placed on the muscle.
- The **BLACK** (-1) lead wire is attached to the electrode in on the lateral aspect **of the gastrocnemius (calf) muscle**. Flex your calf muscle to make sure the electrode is placed on the muscle.
- The **GREEN**(C) lead wire is attached to the electrode on the **lateral side of the ankle** that functions as the ground. Make sure the electrode is placed laterally as you will strike the calcaneal tendon (Achilles Tendon).



Methods Figure 1. Circuit diagram for recording electromyograms from the calf muscles. Make sure the ankle (green) one is on the side of the ankle, the calf (black) is on the lateral aspect of the calf muscle, and the knee (red) is just below and lateral to the popliteal fossa.

Exercise 1: Achilles Tendon Reflex Arc

Objective: To determine the conduction velocity of the Achilles tendon reflex arc.



Procedure:

- Instruct the subject to sit on a tall stool or tabletop so that the subject's thighs are supported by the top of the stool and his or her calves hang freely (feet off the floor).
- The Achilles tendon is located above the heel and connects the gastrocnemius muscle to the tarsal bone of the foot. Tap the tendon smartly with the **wide end of the reflex hammer a few times** to locate a point on the tendon which produces a consistent contraction of the gastrocnemius muscle and a

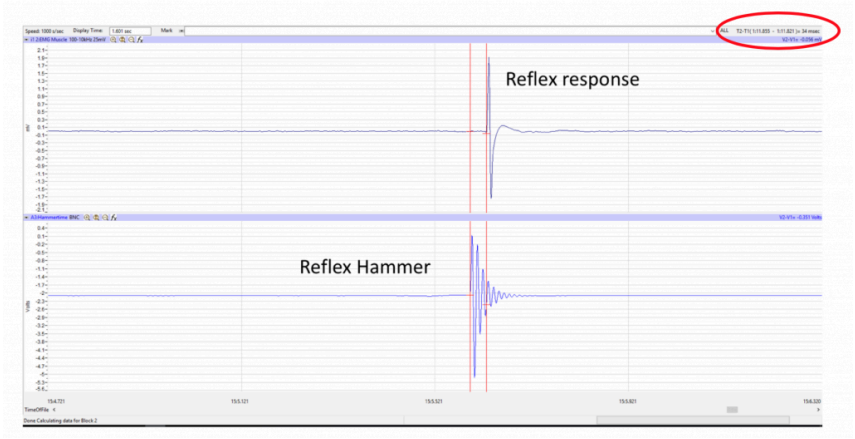
downward movement of the foot (plantar flexion). **Make a mark with a marker at this spot**.

- **Click Record.** Instruct the subject to point his or her toes up and down to demonstrate the type of EMG that occurs during plantar flexion and dorsiflexion at the ankle. Click **AutoScale** on the EMG Muscle (upper) channel.
- Test the hammer by **lightly tapping** on the palm of your hand to make sure the signal is read on the lower "Hammertime" Labscribe screen, only.
- Type **Subject's Name Achilles Reflex** in the Mark box. **Press** the **Enter** key on the keyboard to mark the recording. Continue recording.
- Instruct the **subject to not watch** you, that the exercise has begun, and that his or her tendon could be tapped at any time.
- Adjust the subject's foot so the joint is in neutral position (see photo, below). Hold the foot lightly without applying resistance. Give the subject's Achilles tendon smart tap to elicit the stretch reflex. **Record a total of ten trials** using the same tapping force, noting the plantar flexion of the joint.
- After the tenth trial, **Click Stop** to halt recording.
- Select **Save As** in the File menu, type a name for the file. Designate the file type as *.iwxdata.
- Click on the **Save** button to save the data file.

Data Analysis

Determine the Reflex Reaction Time

- **Scroll** to the **beginning of the data** recorded for the Achilles Reflex to display the first trial on the Main window. You can also click on MARK to show locations for all marks. Highlighting a mark and clicking GO TO MARK will take you to that mark on the data screen.
- Use the **Display Time icons or Zoom Between Cursors** to adjust the display on the **Main window**.

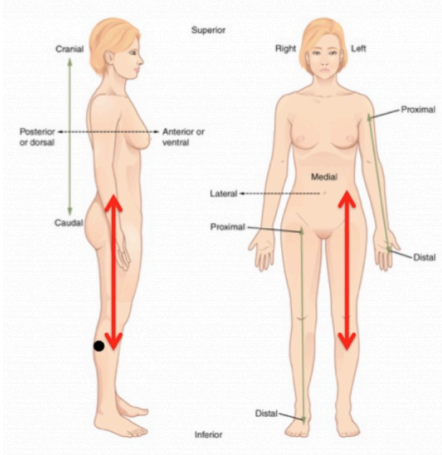


Methods figure 2. An Achilles tendon reflex response and patellar hammer signal displayed on the Main window. The cursors are in position to measure the reflex time.

- Look at the Function Table that is above the display of the EMG channel. The mathematical function, **T2-T1**, should appear in this table.
- Very important: adjust the zoom on the waves, so that you can **clearly see the distinct peaks of the EMG and the hammer tap before it**.
- Drag a cursor to the **beginning of the wave** recorded from the reflex hammer which is displayed on the bottom **Hammertime screen**. Drag the second cursor to the **ONSET** of the **First Wave** on the EMG that is displayed (on top **EMG Muscle screen)** (Methods Figure 2, above).
- Once the cursors are placed in the correct positions for determining the reflex time, record the value for T2-T1 and record this time **on your worksheet.**
- Once the reflex time in the first trial is measured and recorded, move to the data from the **second trial** (tap).
- Repeat the measurement of T2-T1 on the data for all the 10 reflex tap trials by moving the cursors.
- Once the reflex times in all ten trials have been measured and recorded on your worksheet, discard the longest and shortest times from the data set, and determine the average of the eight remaining reflex times.
- Record the mean reflex time for the Achilles reflex in the Data Table in your **Lab Report**.

Determine the Reflex Path Length

Measure the distance between the **belly of the subject's gastrocnemius (calf) muscle (black electrode)** and the site of the sensory-motor synapse in the spinal cord. For the purpose of this exercise, assume that the sensory-motor synapse is at spinal **segment S1,** which is near the iliac spine, or the upper rim, of the hip bone). **Multiply this measurement by 2** to determine the total length of the reflex path. Note the result in meters or mm.



Ask the subject to stand. Measure from the: **belly of the calf muscle** (the black electrode) to **the iliac spine of the "hip bone."** Multiply by 2 to determine the total length of the reflex pathway. **Record on your data table in meters or millimeters.**

Determine the Reflex Conduction Velocity

- Assume synaptic transmission in this pathway takes about 0.5 msec (o.0005 sec), and calculate the conduction velocity in the nerves composing this reflex pathway by the equation:

$$\text{Conduction Velocity (m/sec)} = \frac{\text{Total path length (m)}}{\text{(Mean reflex time (sec)} - 0.0005\text{sec)}}$$

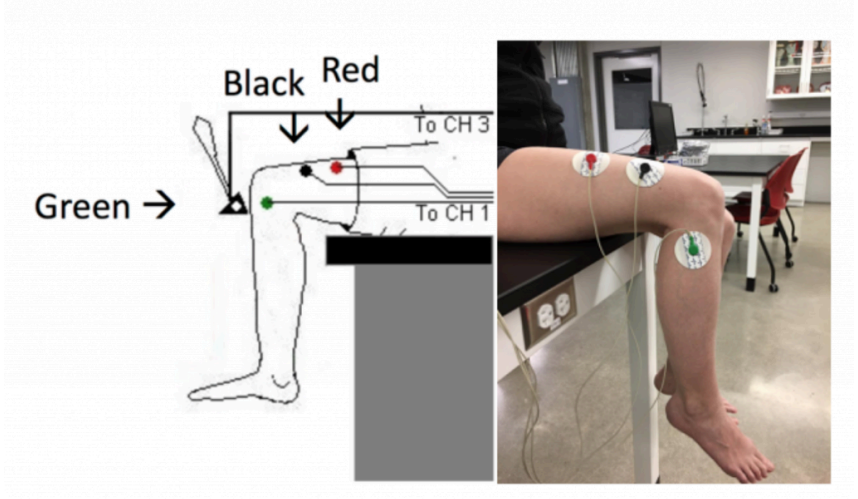
OR

$$\text{CV (m/sec)} = \frac{\text{Total path length (mm)}}{\text{(Mean reflex time (msec)} - 0.5\text{msec)}}$$

- Record the conduction velocity for the Achilles reflex in your Lab Report.
- Save your data to your lab folder, Save As: Student's Name Achilles Reflex.

Exercise II: Patellar Tendon (Knee Jerk) Reflex

Objective: To determine the amplitude and conduction velocity of the patellar tendon reflex arc under different conditions.



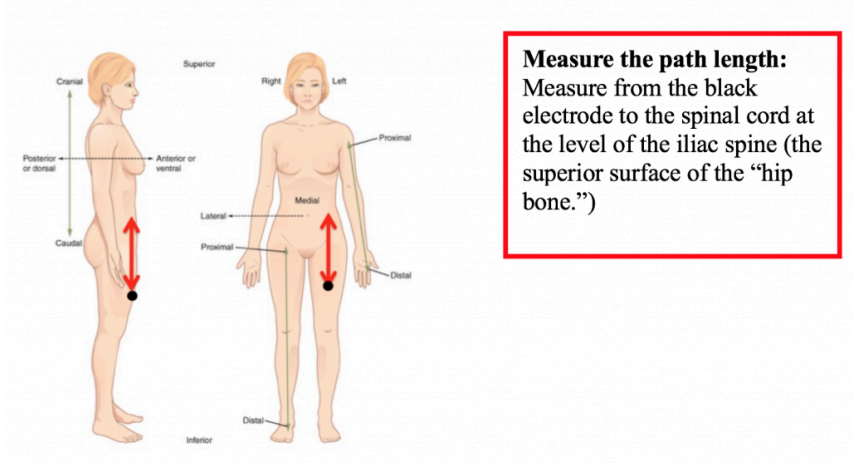
Methods figure 3. Circuit diagram for recording EMGs from the thigh muscles. All electrodes can be on the side of thigh, not directly center. The knee (green) is on the lateral side of the knee not on the kneecap. The lower thigh (black) is 12 cm. above the knee, and the upper thigh (red) is 10 cm. above the black.

Procedure:

- Instruct the subject to **sit on a Tall Stool or tabletop** so that the subject's thighs are supported by the top of the stool and his or her calves hang freely.
- Remove the electrodes over the subject's calf muscle and, if still sticky, place the same set of recording electrodes on the quadriceps muscle of the subject, towards the **lateral side of the thigh (Methods Figure 3)** so that:
- The **BLACK** (-1) lead wire is attached to an electrode which is placed on the "belly" of the Rectus Femoris muscle (flex your quad muscles to locate the muscle) and place the electrode slightly **lateral to the midline of your**

femur.

- The **RED** (+1) lead wire is attached to an electrode which is about 10cm above the negative (black) electrode.
- The **GREEN** (C) lead wire is attached to the electrode on the knee that functions as the ground.
- Measure the Patellar Reflex Path Length from **the black electrode on the quadriceps** to the **iliac spine of the hip (top of the "hip bone"). Double this length for the total length of reflex pathway in meters.** Record on your data table.



Measure the path length: Measure from the black electrode to the spinal cord at the level of the iliac spine (the superior surface of the "hip bone.")

Determination of the Reflex Time for the Patellar Reflex

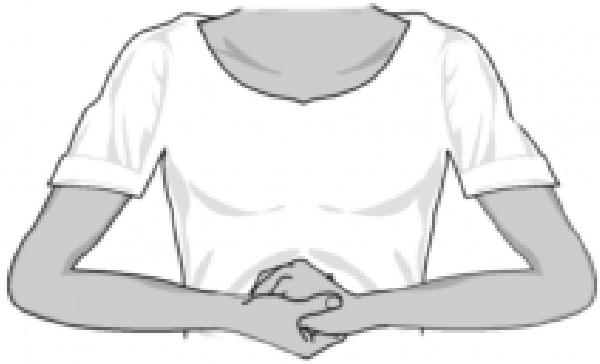
- Feel the position of the patellar tendon just below the kneecap. Place one hand on the patella (kneecap), and use the other hand to tap the patellar tendon with the reflex hammer. Find the point on the patellar tendon that causes the greatest response from the quadriceps muscle. Make a **mark** at that spot.
- **Click Record** and then instruct the subject to raise and lower his or her lower leg to demonstrate the type of EMG that occurs during quadriceps contraction and relaxation. Click **AutoScale** on the EMG screen**.**
- Test the reflex hammer by lightly tapping on your hand to make sure the signal is read on the lower "Hammertime" Labscribe screen, only. **Click Stop** to halt the recording.

- **Type** Patellar Tendon Reflex in the **Mark** box that is to the right of the Mark button.
- **Click Record**. **Press** the **Enter** key on the keyboard to mark the recording.
- **Instruct** the subject to **relax** his or her quadriceps muscle, have the subject **look away** and let them know that the exercise has begun.
- **Tap** the subject's **patellar tendon** to elicit the stretch reflex. **Record a total of ten trials** using the **same tapping force.**
- **After** the **tenth trial**, **click Stop** to halt recording.
- **Select Save** in the File menu.

Reflex Time with Application of Jendrassik Maneuver

To test reflex facilitation, repeat this exercise on the same subject while the subject is performing the **Jendrassik Maneuver** (below). To perform this activity:

- **Mark the data "Jendrassik" in the Mark box so you can find the data recording.**
- The subject should curl the fingers of each hand toward its palm form a cup-shaped grip.
- The subject should hold hands and arms in front of the chest so that elbows are pointed out.
- The subject should interlock the hands using the cup-shaped grip, attempting to pull the hands apart, DURING reflex recording.
- Ask subject to look away and **record 10 trials.**

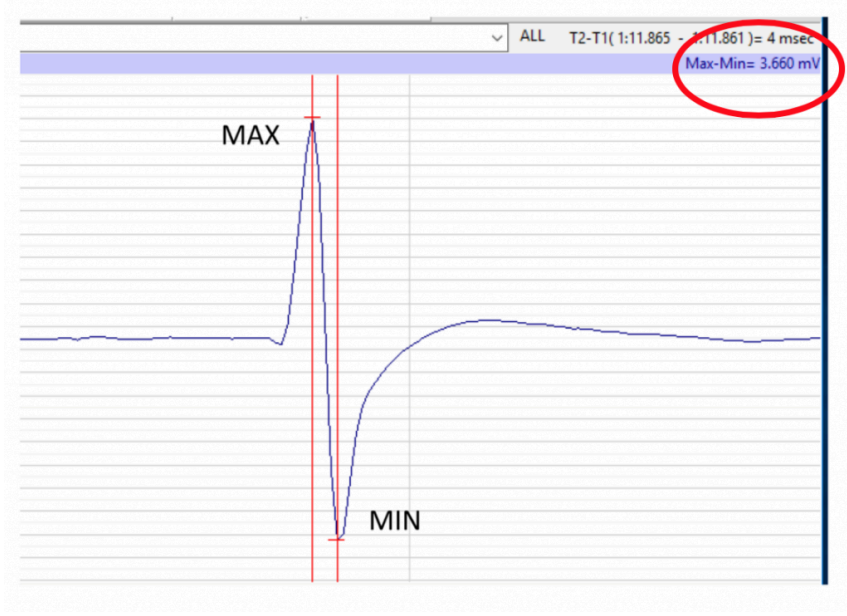


The Jendrassik maneuver

Voluntary Muscle Contraction in Response to Auditory Stimulus

- **To test the response time for voluntary muscle contraction: repeat** this exercise on the same subject (no facilitation), but ask the subject to strongly kick his or her leg in response to **gently tapping the reflex hammer on the lab table**. Note: do not pound the hammer on the table; it will break the hammer!!
    - **Mark the Mark Box "Voluntary Knee Jerk" at the start of the recording.**
    - Ask the subject to look away or close his or her eyes and kick his or her leg in response to hearing the noise of the hammer when it taps the bench top.
    - Lightly tap the hammer on the benchtop at irregular intervals 10 times to **record the 10 trials.**

Data Analysis



Methods Figure 4. Cursor placement for measuring EMG Amplitude (MAX-MIN).

1. Go back to the Patellar reflex data to measure the time and amplitude. In this analysis, you will perform measurements for the patellar reflex, facilitated patellar reflex (Jendrassik), and the voluntary knee jerk conditions.
2. **Measure the response time (T2-T1)** following the instructions in Exercise 1 for cursor placement. After you have the reflex time measurements go on to Step 3 below.
3. Measure the **EMG amplitude (Max-Min; see the red circle on Methods Figure 4)** with double cursors on the **EMG Screen**:

    - Using the arrow keys on your key board helps to move the cursor more precisely when you are very close to wave peaks.
    - Place the first cursor at peak of the **first EMG wave**
    - Place the second cursor on the **bottom of the same EMG wave**
    - Record **Max-Min** amplitude in mV on your Worksheet. **Calculate the average** in your data table on the Lab Report.

Citations

- Reflexes by Janet Chen Daniel. Last accessed January 22, 2020. http://csmbio.csm.jmu.edu/biology/danie2jc/reflex.htm
- Disorders of the nervous system by Reeve and Swenson accessed at https://www.dartmouth.edu/~dons/part_1/chapter_8.html
- Passmore SR, Bruno PA. Anatomically remote muscle contraction facilitates patellar tendon reflex reinforcement while mental activity does not: a within-participants experimental trial. *Chiropractic & Manual Therapies*. 2012;20:29. doi:10.1186/2045-709X-20-29.

HUMAN NERVE CONDUCTION VELOCITY (NCV)

The NCV Test

Karri Haen Whitmer

Background

Conduction of an impulse in human nerves relies upon the electrochemical activity of the individual neuron fibers inside the nerve. Each fiber (axon) inside the nerve is capable of propagating an **action potential** if the stimulus is strong enough to bring the membrane potential of the neuron to the threshold value. Depending on the strength of the stimulus, not all fibers in a nerve may fire. Despite this, the action potential is an all-or-none phenomenon, where it will always have the same amplitude and time course for a single neuron. When action potentials are recorded via recording electrodes on the skin, the result is a "compound action potential," which is an algebraic sum of action potentials from many cells. We have already observed **compound muscle action potentials (CMAP)** in the EMGs of previous labs.

Signal transmission within and between neurons

The action potential is the converse of the cell membrane resting potential, which, for neurons registers -70 mV on the voltmeter. The action potential involves several steps, starting with the onset of a stimulus. Most of the time, stimuli generating action potentials are chemical, where a ligand, a **neurotransmitter**, binds to a **ligand-gated ion channel** in the cell membrane of the dendrite or cell body of the neuron. If the graded potential is excitatory and sufficiently strong, the dissipation of charge across the cell body membrane reaches the **axon hillock** and depolarizes the cell membrane to -55 mV. The axon hillock is a region of high voltage-gated ion channel density.

Voltage gated channels open in response to **threshold depolarization** of the membrane, leading to an influx of sodium (via the voltage gated sodium channel), bringing about the onset of **Phase 1** of the action potential (Figure 1). The influx of sodium drives the membrane potential to +30 mV, which is the peak of the action potential on a graph. From here, voltage gated potassium channels, which slowly open near the end of Phase 1 of the action potential, allow efflux of potassium from the cell. The membrane repolarizes in response to the efflux of potassium, and the membrane potential reaches resting potential at the end of **Phase 2**. However, because the potassium channels close very slowly compared to sodium channels, the continued leakage of potassium from the cell results in membrane hyperpolarization, during which the membrane potential reaches -90 mV. At the end of phase 3, the activity of the **sodium/potassium ATPase** active transporter, which exports three sodium ions and imports two potassium ions helps to bring the membrane potential back to -70 mV (along with passive sodium and potassium leak channels).

In order to communicate the signal of the action potential to another cell, **synaptic transmission** must occur. Synaptic transmission is the rate-limiting step of neural transmission, meaning that it is the slowest part of signal transmission. During synaptic transmission, the action potential reaches the axon termini of the pre-synaptic cell. The influx of sodium depolarizes the membrane of the termini and opens **voltage gated calcium channels**. Calcium enters the cell, resulting in the fusion of neurotransmitter-filled vesicles with the pre-synaptic membrane.

Neurotransmitter diffuses across the **synaptic cleft** and binds to a receptor on the post-synaptic cell. The binding of neurotransmitter to a receptor will cause a **post-synaptic potential (PSP)** (a graded potential). Post-synaptic potentials may be excitatory or inhibitory, depending on the interaction of the neurotransmitter with the receptor.

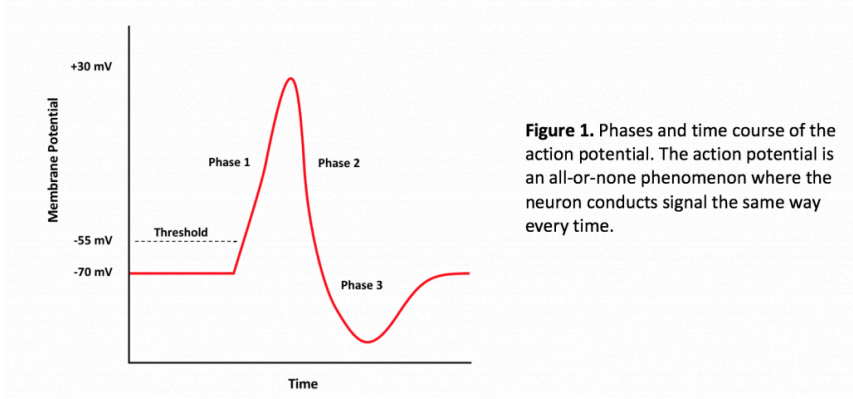


Figure 1. Phases and time course of the action potential. The action potential is an all-or-none phenomenon where the neuron conducts signal the same way every time.

Figure 1. Phases and time course of the action potential.

Speed of neural transmission

The speed of neuron transmission generally depends upon two factors: axon size and degree of myelination. Larger diameter axons transmit signal faster due to lower resistance to the flow of charge inside the axon. Myelinated axons (white matter) transmit action potentials faster, up to approximately 150 m/s, due to saltatory conduction (Figure 2). In **saltatory conduction**, ion channels are only necessary between regions of myelination (Nodes of Ranvier); thus, the signal dissipates quickly under regions of myelination to each node and is re-established there, until the signal reaches the terminus. On the other hand, in unmyelinated axons (gray matter), conduction is continuous. This means signal transmission is relatively slow because the voltage gated ion pumps must be present, and used, on all regions of the axonal membrane. Continuous conduction speeds rarely reach 10 m/s. **Nerve conduction velocities**, which are calculated as the result of the transmission of hundreds or thousands of action potentials firing along the axons of neurons inside nerves, may be affected by the health of the nervous system, age, sex, and even temperature.

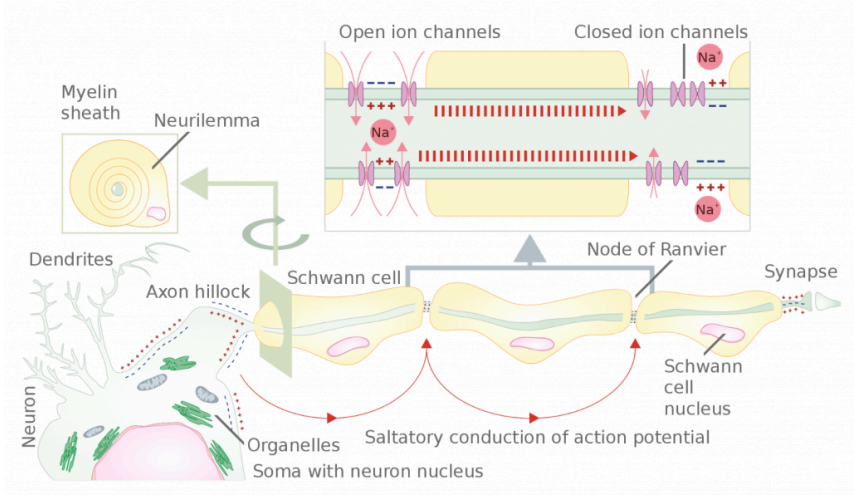


Figure 2. Saltatory conduction of an action potential by skipping node-to-node. In salutatory conduction, transmission speeds are higher than in unmyelinated neurons because the signal quickly "jumps" between nodes of Ranvier, regenerating at the voltage gated sodium channels of each node. Image CC by SA 4.0.

Disease and neural transmission

Electromyograms (EMGs) are a non-invasive way to determine nerve conduction velocities in the clinical setting. In one type of **nerve conduction velocity test (NCV)** an EMG recording electrode detects the contraction of a muscle in response to direct neural stimulation by a **stimulating electrode** placed upon the skin. The time from the onset of stimulus (firing of the stimulating electrode) until muscle contraction is recorded. The stimulating electrode is then moved and the measurement is taken again. From this information, one can determine the nerve conduction velocity.

Nerve conduction velocities inform us about the relative health of peripheral nerves. Damage to nerves or diseases that cause demyelination (such as multiple sclerosis – MS) or nerve degeneration (like amyotropic lateral sclerosis – ALS) are primary concerns when conduction speeds are undetectable or below the normal range. The peripheral nerves of the arms generally have conduction velocities between 50-60 m/s.

Interpreting EMGs resulting from direct electrical stimulation of a nerve

Because there are both sensory and motor fibers in the Ulnar nerve, both will be stimulated upon application of stimulating electrodes to the skin. Activation of sensory fibers will initiate the "H-reflex," for which the pathway includes the sensory neurons, integrating center (spinal cord), and motor neurons. This is much like we saw last week in the muscle spindle reflex experiments. Application of current to the ulnar nerve also results in an M-wave, an EMG wave due to direct activation of the motor neurons, which will quickly produce a muscle response. In this lab, we will take measurements from the M-waves, only. Note that passive transmission of current in body tissues from the stimulating electodes also produces a stimulus artifact on the EMG. Please see the figure below (Figure 3).

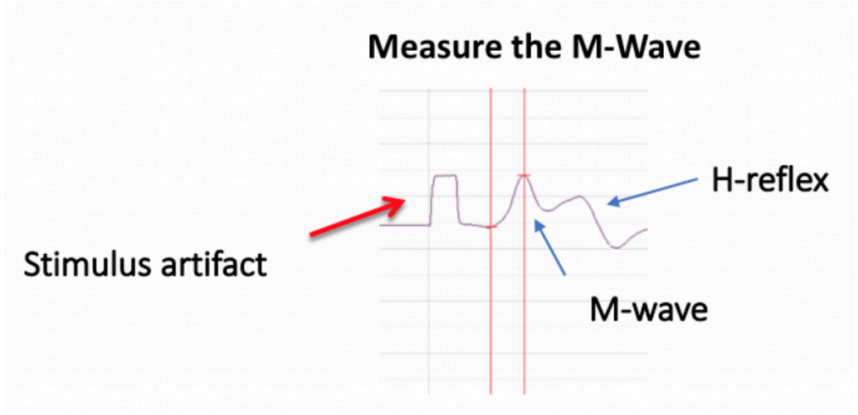


Figure 3. During application of optimal stimulus with the stimulating electrodes, a stimulus artifact, M-wave, and H-reflex will be apparent on the EMG. Measure the M-wave, only, in this week's experiments.

This week's experiment:

This week, we will perform one experiment (lab exercise 1) and one clinical evaluation (lab exercise 2). The experiment will determine the effects of **increasing stimulus strength** on the EMG. Stimulus (measured in milliamperes, mA) is applied directly to the fibers of the **ulnar nerve** with **stimulating electrodes** on the skin. The resulting **EMG amplitude** is recorded in mV. EMG amplitude measures

the strength of contraction by the innervated muscle, which is relative to the number of **motor units** activated by the stimulus. Recruitment of more motor units (activation of additional motor neurons and their innervated muscle cells) is accomplished by increasing the stimulus.

Next, we will perform a **nerve conduction study** using EMG. Here, we will use the optimal stimulus strength from Experiment 1 to re-stimulate the ulnar nerve. After recording the location of the stimulating electrode on the arm and obtaining the EMG, the stimulator will be moved to a secondary position, re-stimulating the ulnar nerve. Here, we will divide the physical distance between the stimulators (in mm) by the difference in time between the two trials' M-waves on the EMG. This method of calculating conduction velocity is called the **difference method.**

Measurements in this lab:

In this week's lab, we will measure the effect of stimulus strength on the amplitude of the compound muscle action potential visualized on the electromyogram. We will then use the information from our experiment to determine the conduction velocity (transmission speed) of the ulnar nerve in a nerve conduction study.

To help prepare for this week's lab, answer the following:

1. What is the dependent variable tested in this week's experiment (lab exercise 1)?
2. What independent variable is tested in this week's experiment (lab exercise 1)?
3. Can you think of an experiment you could conduct using the methods of the nerve conduction study (lab exercise 2)? *Hint: what kinds of variables might affect the speed of nerve transmission?*

Laboratory Methods

Laboratory Methods Highlights

· This week, instead of stimulating a nerve with a reflex hammer via stretch receptors, we will stimulate a nerve directly with stimulating electrodes.

· Please wear short sleeves or sleeves that can be rolled above the elbow, half way up the brachium.

· A stimulating electrode delivers a mild electric shock, which, if sufficiently strong, depolarizes the membranes of neurons, causing them to fire.

· Neurons stimulated with stimulating electrodes behave like other neurons, except that when stimulating the fibers of a nerve, we may provoke action potentials in both sensory and motor fibers at the same time.

· In this lab we will stimulate the ulnar nerve and determine the outcome of increasing stimulus strength (in mAmps).

· Later, we will re-stimulate the ulnar nerve from two different locations along the arm in order to determine the conduction velocity.

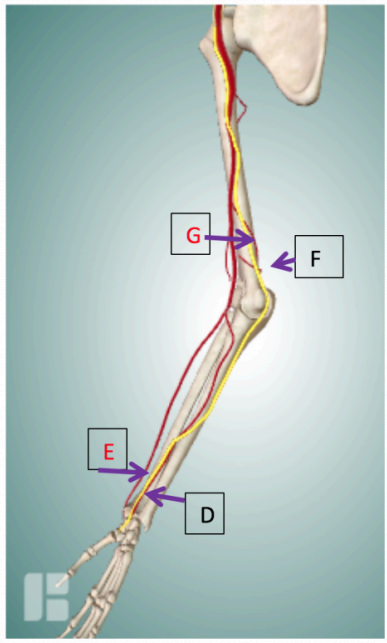
Equipment Required: IXTA data acquisition unit, iWire-B3G cable and three EMG lead wires, disposable snap electrodes, HV stimulator lead wires.

I. Equipment Setup: Start the Software

Turn on the iWorx hardware box at the switch on the back, and select the Week7_NerveConduction.iwxset file from the P-drive.

II. Equipment Setup: Electrode Placement

1. The subject should remove all jewelry from his/her left or right hand and wrist.
2. Study the pathway of the ulnar nerve in the picture (below).

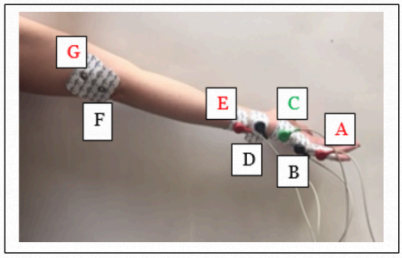


Note how the ulnar nerve starts a path towards the posterior of the forearm. The area just distal to that you will be placing Stimulator electrodes for Exercise 1:
D (Black Stimulator)
E (Red Stimulator)

And then for Exercise 2:
F (Black Stimulator)
G (Red Stimulator)
These electrodes may have to be placed on the medial side of the distal triceps area
As everyone may not have an identical nerve path, placement of these electrodes may vary between students.

Methods Figure 1. Ulnar Nerve Pathway. The ulnar nerve conduction velocity will be assessed in this experiment. Ulnar Nerve Pathway. The ulnar nerve conduction velocity will be assessed in this experiment.

3. Clean the areas where the electrodes will be attached with an alcohol pad. Lightly abrade the skin in those areas.



Trim electrodes for placement. Keep plastic backing on them when cutting, to prevent adhesive build up on scissors.

Electrode placement for Exercise 1. A B C (EMG leads)
D & E (stimulator leads)

Methods Figure 2. Placement of the electrodes on the medial edge of the left or right hand and arm following the path of the Ulnar Nerve.

4. Obtain **Seven** disposable electrodes. Label **5** them on the pointed tab with the letters **A through E.**
5. **Cut down** the electrodes so just the Medline logo shows. **Keep the plastic backing on them to reduce adhesive build up on the scissors.**

- Place **A** on the medial edge of the little finger of the right hand, so the electrode button/snap is just above the first knuckle. This is for the **RED** (+) recording electrode in Figure HN-3-S2.
- Place **B** on the **medial edge of the palm**, so the electrode button/snap is **at the base of the little finger.** This is for the **BLACK** (-) recording electrode on the hand.
- Place **C** so the electrode button/snap is on the **medial edge of the wrist just above the crease** of the wrist. This is for the **GREEN** ground electrode on the hand.
- ***Warning: Before connecting the IXTA stimulating electrodes to the subject, check the Stimulator Control Panel in the LabScribe toolbar to make sure the amplitude value is set to zero (0 AMP).***

Next, attach the remaining 2 electrodes to the subject's arm so that they are placed on the pathway of the Ulnar Nerve in the following configuration:

- Place **D** the medial edge of the forearm, so the electrode button/snap is above the wrist crease. Attach the **BLACK (-) stimulating electrode.**
- Place **E**, the positive stimulating electrode, right above the negative stimulating electrode towards the medial side of the forearm. Attach the **RED (+) stimulating electrode**.
- Electrodes F and G (in the photo) will be placed later for Exercise 2.
- **Videos of proper electrode placement for Exercises 1 and 2 are found on the P-Drive.**

Safety precautions for this lab:

You will deliver mild electric shocks to either yourself or a volunteer experimental subject. The equipment you use to do this is carefully designed to keep the parameters of the electroshocks well within a safe range, and this experiment is safe

and fun. Nevertheless, we ask that you place stimulating electrodes on the arms **only**, and only place electrodes on the same side of the body **(never on both arms at the same time).**

If you think you may be pregnant or if you suffer from known heart conditions or have an artificial heart pacemaker please do not volunteer as an experimental subject this week.

Exercise 1: Stimulus Strength and Muscle Response

Objective: To determine the effect of stimulus strength on the response of the innervated *abductor digiti minimi* muscle.

Overview: You will stimulate the abductor digiti minimi motor neuron with increasing amounts of current (measured in milliAmps, read as AMP on LabScribe). You will measure the EMG muscle response each time (in mV) and record the value in your lab report. You will continue until you get three readings in a row with the same value (+/- 0.2 mV). Of those three readings, put a mark next to the lowest current (Amplitude) value on your lab report; you will be using this value for exercise 2.

Checking electrode placement and preparing to record data

1. Ask the subject to place his or her right hand on the bench with the palm down or let hang relaxed at their side. Tell the subject to relax. ***Note:*** *The subject should make sure to relax his/her forearm and hand completely. At the beginning, you will need to continue to increase the pulse amplitude until a response is generated. Stimulus that does not create a muscle contraction is subthreshold.*
2. When starting this experiment, check to make sure 0 is the number in the AMP Window. If it is not, please change the value to zero. **Click Record** button on the LabScribe Main window. LabScribe will record a single sweep with a display time of **50 milliseconds**. Since the output amplitude is set to

zero, there should be no response from the *abductor digiti* muscle (no muscle twitch in the pinky finger).

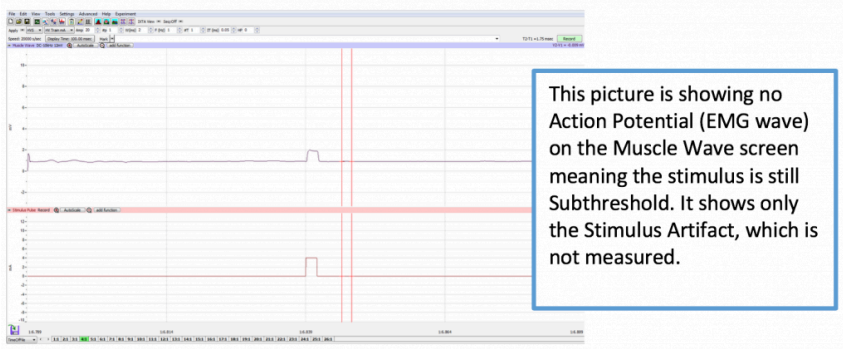
3. Increase the output **amplitude to 1** milliAmp on the Tool Bar by using the up/down arrows or placing 1 in the box by highlighting and changing the number to 1 (AMP). **Click "APPLY"**. **Click the Record** button again and record another sweep. Click the AutoScale button for the Muscle channel to improve the display of the muscle's response (Methods Figure 1).

Continue to **increase the output amplitude by an increment of 1 mAMP (1 "AMP" on the LabScribe stimulator button) until you get a muscle twitch and EMG M-wave for the subject. Click apply.**

Remember, after each increase in amplitude, you must ***click "APPLY" before*** *doing another recording!*

- 1 AMP – APPLY – Record
- 2 AMP – APPLY – Record
- 3 AMP – APPLY – Record
- 4 AMP – APPLY – Record...etc.

- **If you get no Action Potential Wave on the EMG screen by 10 AMP,** reposition the electrode pads starting with **D.**
- Keep the snaps in line and move **D & E slightly to one side of the previous position.**
- **Ask your TA for help.**



Methods Figure 3a.

Methods Figure 3b. Muscle response to stimulation of the ulnar nerve shown in the LabScribe software.

Start data collection for Experiment 1

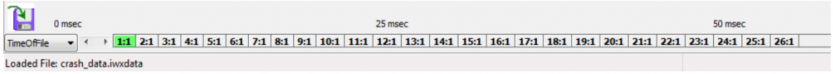
- Now that we know the electrode placement is optimal, open a new data file. EACH PARTICIPANT should have their own data file for this lab.
- Go back to AMP 1 to start collecting data.
- After recording each "sweep" (each time you click RECORD), **immediately perform the data analysis on the Main Window (see below). Do not**

record all sweeps and go back to do analysis, as it may make longer.

- Continue to increase the output amplitude by 1 AMP always **clicking "APPLY"** and then recording the response **until the muscle impulse reaches a maximum (the amplitude of the M Wave peaks from your analysis no longer increases with additional applied stimulus):** once you get **three readings in a row with the same value (+/- 0.2 mV),** note the lowest of the three AMP values. You will be using this value for exercise 2. **A maximum of 19 is possible; do not go past 19 AMP.**
- When you are done, be sure to select **Save As** in the File menu and name the file. Choose a destination on the computer in which to save the file (e.g. your folder). Click the Save button to save the file (as an *.iwxdata file).

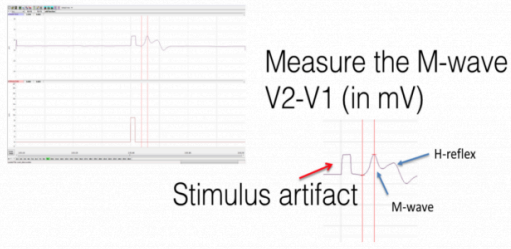
Exercise 1 Data Analysis

Different sweeps may be selected any time by clicking on the Sweeps list on the bottom of the Main Screen (1:1, 2:1, etc.). If analyzing data on the Main Screen while recording, the sweep you need to analyze is already selected. The Sweeps task bar is shown below.



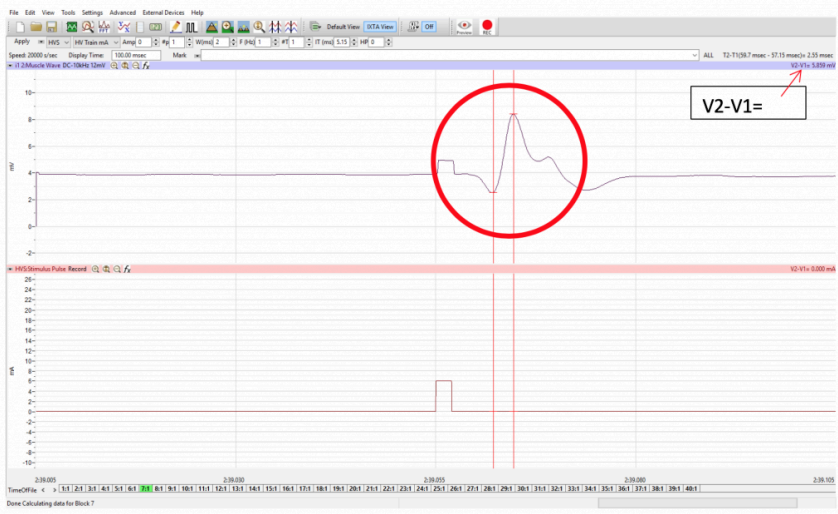
1. Record V2-V1, the M-Wave amplitude

    - **To find the amplitude:** Click and Drag one cursor to the base of the EMG wave and the second cursor to the top peak EMG wave**. In the case of multiple waves select the first EMG wave (not the stimulus artifact), the M-wave is shown in the picture, below.** The value **V2-V1** found in the table at the top right of the Main window is the amplitude of the muscle response. Record the amplitude of each response (in mV) with its corresponding applied stimulus strength (in mA) **in the table in your lab report**. Measure all visible EMG Waves (action potentials). If there is no EMG M-Wave record "Subthreshold" on your lab report.

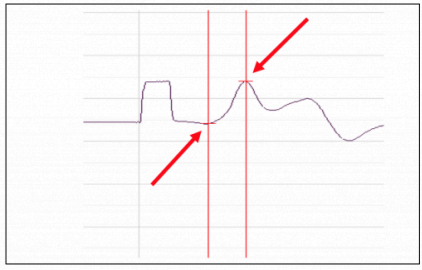


Methods Fig. 4. The first plateau-like wave is a stimulus artifact from the stimulator electrodes, the second wave is the M-wave (muscle wave) that should be selected and recorded, the third wave is a secondary reflex due to stimulation of the sensory fibers of the nerve (H-reflex).

Methods Figure 4A. Measure the M-Wave V2-V1 (in mV).



Methods Figure 4B.



Make sure that the cursor crosshairs are at the very bottom of the 1st EMG Wave and the second cursor is at the very top of the peak of the same EMG wave.
In the case of double waves, select the first EMG wave (the M-wave).
Only perform measurements on the top screen labeled Muscle Wave.

Methods Figure 4C.

Exercise 2: Ulnar Nerve Conduction Velocity

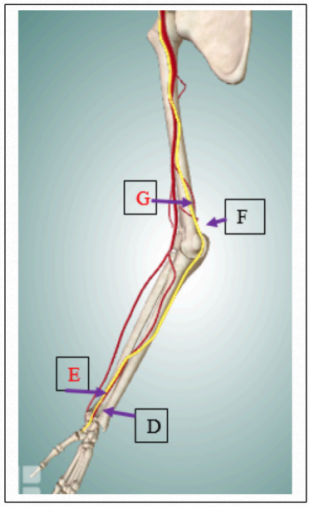
Objective: To measure the conduction velocity (transmission speed) of the ulnar nerve.

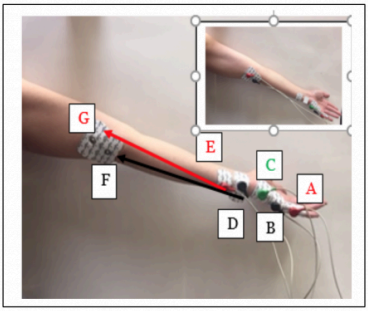
Overview

You will **use the AMP value which excited the maximal response in experiment 1** and stimulate the same *abductor digiti minimi* motor neuron further up the arm. You will then use that previous experiment and the one you will conduct here to **compare the time between innervation and the elicitation of the response for two points on the nerve**. You will measure the distance between the stimulation electrodes and calculate the transmission speed in m/s.

Procedure

1. Ask the subject to place his or her right hand on the bench with the palm down. Tell the subject to relax.
2. Move the **red (+)** stimulating lead from Electrode E to Electrode G, and **the black (-)** stimulating lead from **Electrode D to Electrode F.** Video available on the P-Drive.



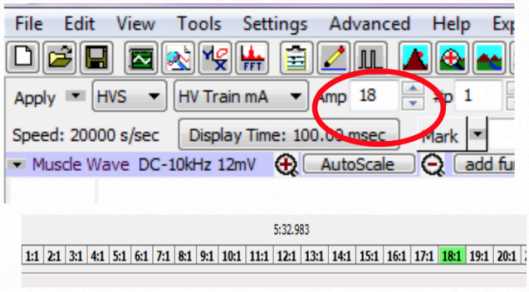


Methods Figure 5. The placement of electrodes **F Black Stimulator Lead** just above the medial epicondyle of the humerus as shown in the picture on the left.
The placement of electrodes **G Red Stimulator Lead** just above the placement of **F Keep the snaps for the attachment of the leads in line. Both should be placed on the upper Ulnar Nerve.**

Methods Figure 5.

Test the placement by setting the Amplitude to the number that produced the optimal Action Potential previously. Click Apply and Click Record. If you get an Action Potential on the EMG screen proceed to Step 1 below.

If you do not get an Action Potential, reposition **F** and **G** electrode pads and test again. Ask your TA for help. Reposition the stimulator electrodes until you get an Action Potential then proceed to step 1 below.



Select the optimal stimulus strength from Exercise 1. This is the value at which the M-wave amplitude no longer increased. (May be less than 18, shown here.) Change the value in the Amp window to this value for Exercise 2.

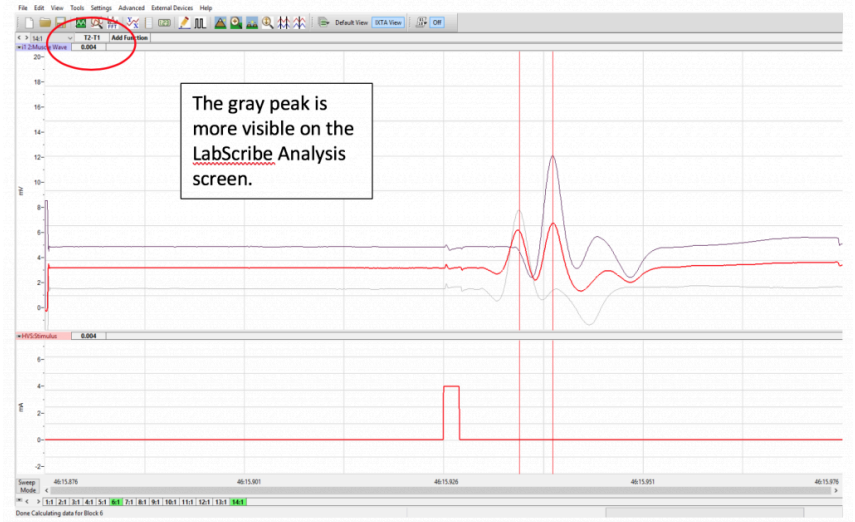
Methods Figure 6.

1. Set the Amplitude on the Labscribe toolbar to the first AMP value that delivered a maximal muscle response in Exercise 1. **Click Apply.**
2. **Click the Record** button on the LabScribe Main window to record a single sweep at this stimulus strength.
3. Select Save in the File menu.
4. Use a tape measure and measure the actual distance, in millimeters, between the placement of the black (-) stimulating electrodes (Electrodes **D** and **F**) in exercises 1 and 2: Measure from the center of the snap on **D** to the center of the black stimulator on **F,** in a straight line.

Exercise 2 Data Analysis

1. Click on Double Cursors.
2. **Click the Analysis** icon in the LabScribe toolbar to view the recorded sweeps. From the Sweeps list, select the sweep that had delivered the **maximal muscle response in Exercise 1, along with the sweep from Exercise 2**. This will superimpose the sweeps on each other (see Methods Fig. 6, below). The Red line is the algebraic sum of both, ignore that line.
3. Now measure peak to peak by placing one cursor on the **M-wave peak of the gray line** and the other cursor on the **M-wave peak of the black line**. If the cursors do not move and stay where you want them Click the Double Cursor again.
4. If T2-T1 are not on the Tool Bar, select **add function** on the Tool Bar, then **General** on the drop-down menu and then select **T2-T1**.
5. The value for T2-T1 displayed in the Tool Bar on the Analysis window is the difference in response time between the two positions of the negative stimulating electrode (e.g. it takes longer to elicit the M-wave the further the stimulating electrodes are from the muscle being measured). Note that the time on the Analysis Tool Bar is in seconds and you need to convert it to milliseconds. (example, 0.004sec = 4ms)
6. Calculate the **conduction velocity (in m/sec)** by dividing the distance (in mm) between the two positions of the negative stimulating electrode by the time (T2-T1) between the peaks of the muscle responses from those two positions.
7. For example:**225 mm between positions of (-) electrode / 4 ms =**

56.25mm/ms = 56.25m/s Report the velocity and show all your calculations on your lab report.



Methods Figure 6. Cursors mark the time difference between the peaks of the muscle responses caused by stimulating electrodes at two different positions on the ulnar nerve.

AUDITORY AND VISUAL PATHWAYS

Reaction Times

Karri Haen Whitmer

The nervous system allows us to detect changes in the environment and react to them. An external stimulus is detected by a receptor, which sends sensory information to the central nervous system, where it is processed. If a motor response is initiated, it usually involves a series of action potentials which produce muscle contraction and movement in one or more parts of the body. A reflex is the most simple stimulus-response reaction. A loud sound or something flying at your eye makes you blink, while a tap on the tendon under the knee cap produces the knee-jerk (or myotactic) reflex.

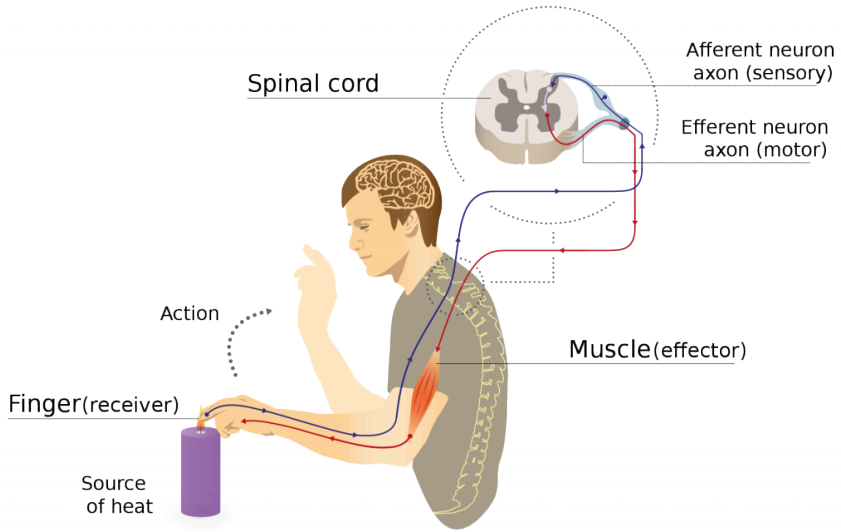


Figure 1. A cross-section of the spinal cord showing the single synapse between the sensory and the motor neurons involved in a myotactic reflex. Image by Marta Aguayo **CC BY 3.0.**

A simple reflex like the myotactic reflex is produced via single synapses between sensory axons and motor neurons. The required circuitry for this reflex is confined to the spinal cord, as shown in Figure 1. Sensory information also ascends to higher centers, but the brain is not necessary or required to perform the reflex. More complex reflexes usually involve additional (inter-) neurons and more than one population of motor neurons. Thus, more neurons and synapses are involved, which usually results in a longer delay between stimulus and response and often a more complex response. One example of such a complex response is the flexion withdrawal reflex, where a noxious stimulus to one leg causes withdrawal of the stimulated leg and extension of the other.

Visual and Auditory Reflexes & Reaction Times

Now that we understand neural transmission and synapses involved in basic reflexes, we can begin to ask how nerve cells are assembled into more complex structures like pools, circuits, and pathways. Stimulus on the nervous system exerts its effects on special cells called receptors. These cells are modified to respond to "outside information" rather than to synaptic inputs from other nerve cells. Outside information can take the form of light entering our eyes; of mechanical deformation to cells in the cochlea or vestibule due to sound or pressure waves; or of chemicals, as in our sense of smell or taste. In all these cases, the effect of the stimulus is to produce an electrical signal in the receptors and consequently a modification in the rate of neurotransmitter release at their terminals.

The visual and auditory systems work separately and in combination with each other to inform and guide the body's internal and external actions. Sometimes responses to light or sound are simple and are considered reflexes. Other pathways contain more neurons and require more processing in the higher brain centers.

Auditory reflexes encompass reflexes initiated by auditory stimuli. When focus is required, auditory reflexes instantaneously block out unnecessary sound frequencies, and when general auditory awareness is required, the auditory reflexes expand sound frequency access to ensure the central nervous system is provided with all the information it needs to respond appropriately. Other auditory reflexes involve

involuntary motor responses to sound, such as the orientation reflex (involuntary head turn for locating the source of an unexpected sound) and the startle reflex (an inborn protective reflex).

The visual system distinguishes variations in shape, color, brightness, movement, helping to distinguish familiar people, places and things from unfamiliar, to determine relative location, and detect visual input important to daily function and general survival. Visual reflexes adjust instantaneously from static and dynamic visual input that is near or far, blocking out extraneous visual input when visual concentration and focus are required, while remaining vigilant to unusual visual input important to productive functioning and general safety. While the visual and auditory sensory systems each provide the body access to unique forms of stimulus input, they also work together to coordinate "seeing-hearing" information and with each of the other sensory systems to inform and prioritize input for the central nervous system to guide and direct action in response to ever-changing conditions. Due to a number of issues the auditory and visual systems can become hypersensitive or hyposensitive or simply not function.

Neural Pathways for Vision

Perception of incoming light begins when the photons are detected by the retina's **photoreceptors** (rods and cones). In the most-simple pathway, photoreceptors synapse with bipolar cells, which then synapse with the retinal ganglion cells that exit the back of the eye as the optic nerve (cranial nerve II).

The two optic nerves exit each eye at the optic disk and combine at the base of the brain just in front of the brainstem to form the **optic chiasm (Figure 2)**. In the optic chiasm, half of the axons from each eye cross over, or decussate, to the other side of the brain. Note in the figure below that input from the left visual field strikes the nasal retina (side closest to the nose) of the left eye and the temporal retina (side closest to the side of the head) of the right eye. Likewise, input from the right visual field strikes the nasal retina of the right eye and the temporal retina of the left eye. Therefore, both eyes receive information from both visual fields.

In the optic chiasm, axons originating from nasal ganglion cells cross to the opposite side, whereas axons originating from temporal ganglion cells stay on the side of

origin. The result is that after the optic chiasm, all input from the right visual field travels in axons in the left side of the brain, and all input from the left visual field travels in axons in the right side of the brain.

Although the axons are still those of ganglion cells, after the optic chiasm the axons travel in what is called the **optic tract**. The ganglion cells terminate in a nucleus in the thalamus called the **lateral geniculate body**, where they form synapses with neurons that ascend to the primary visual cortex in the occipital lobe. Pathways from the lateral geniculate body to the visual cortex on either side are called the **optic radiations**.

To simplify the visual pathway, we note that photoreceptors synapse with 1) bipolar cells in the retina. Retinal bipolar cells synapse with 2.) retinal ganglion cells. From here, the axons of ganglion cells may decussate at the optic chiasma or remain on the ipsilateral side and synapse with 3.) neurons of the lateral geniculate body of the thalamus, which form optic radiations. 4.) The optic radiations synapse on the primary visual cortex (V1) in the occipital lobe of the brain, which is physically very far from the photoreceptors of the retina. You can approximate this distance by measuring from your eyes to the occipital region of the skull. *For conscious awareness of visual information*, additional processing must take place in the brain cortex, including several visual processing areas (V1-V6), which add many other synapses to this pathway.

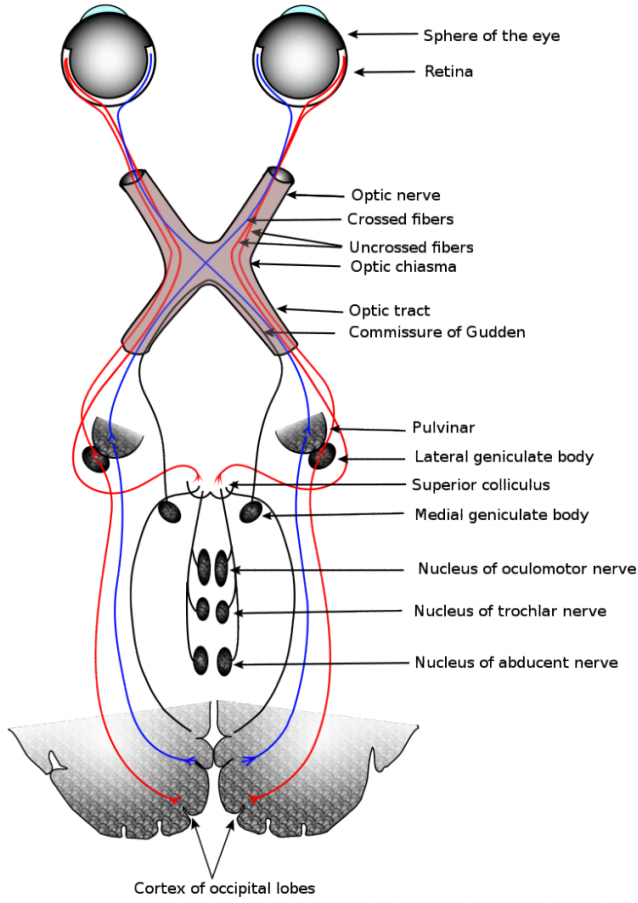


Figure 2. The optic pathway. Axons of ganglion cells exit the posterior eye to become the optic nerve. Medial fibers cross at the optic chiasm and create the optic tracts. Optic radiations synapse with regions of the visual processing cortex.

Neural Pathways for Sound

Sound, or disturbances in the distribution of air molecules, is converted into pressure waves inside the cochlea. As the pressure waves vibrate the cochlear membranes, the tectorial membrane of the cochlear duct contacts and bends the stereocilia of auditory hair cell receptors.

When hair cells are bent, the neurotransmitter, usually glutamate, released from hair cells binds to receptors on afferent neurons of the cochlear nerve, part of cranial nerve VIII. The hair cell transmitter depolarizes the afferent neuron. The greater the degree of depolarization, the greater frequency of action potentials in the afferent neuron, which therefore codes for the intensity of the sound.

The afferent neurons of the auditory nerve terminate in the **cochlear nuclei** in the brainstem. From here, the auditory pathway can be complex. The simplified figure (below), shows secondary neurons passing through the **auditory reflex centers of the inferior colliculi** and synapsing with third-order neurons that travel to a nucleus of the **thalamus** called the **medial geniculate body**. In the medial geniculate body, the third-order neurons form synapses with quaternary neurons that transmit information to the **auditory cortex** in the temporal lobe, resulting in the conscious recognition of sound.

To simplify the auditory pathway: 1.) hair cell receptors synapse with a cochlear branch of the vestibulocochlear nerve. 2.) These fibers then terminate in the cochlear nucleus and synapse with secondary neurons that ascend to the inferior colliculus. 3.) Tertiary neurons extend to the thalamus and synapse with 4.) quaternary neurons that project into the auditory cortex. Note that the overall path length of the auditory pathway is relatively short because the inner ear is proximal to the temporal lobe of the brain, and relatively little additional cortical processing is required for the conscious recognition of auditory stimulus (short pathway, few synapses).

In this laboratory, we will examine the reaction times for auditory and visual responses to stimulus. The reaction time is simply the time required for processing between a visual or auditory stimulus and a response**.** We can compare this information to our anatomy knowledge of the number of synapses in a pathway, and the path length, to determine what type of information is processed faster by the human body. Reaction time measurements will be taken from an individual subjected to harmless visual and sound stimuli. In addition, the effect of priming and prediction may be examined.

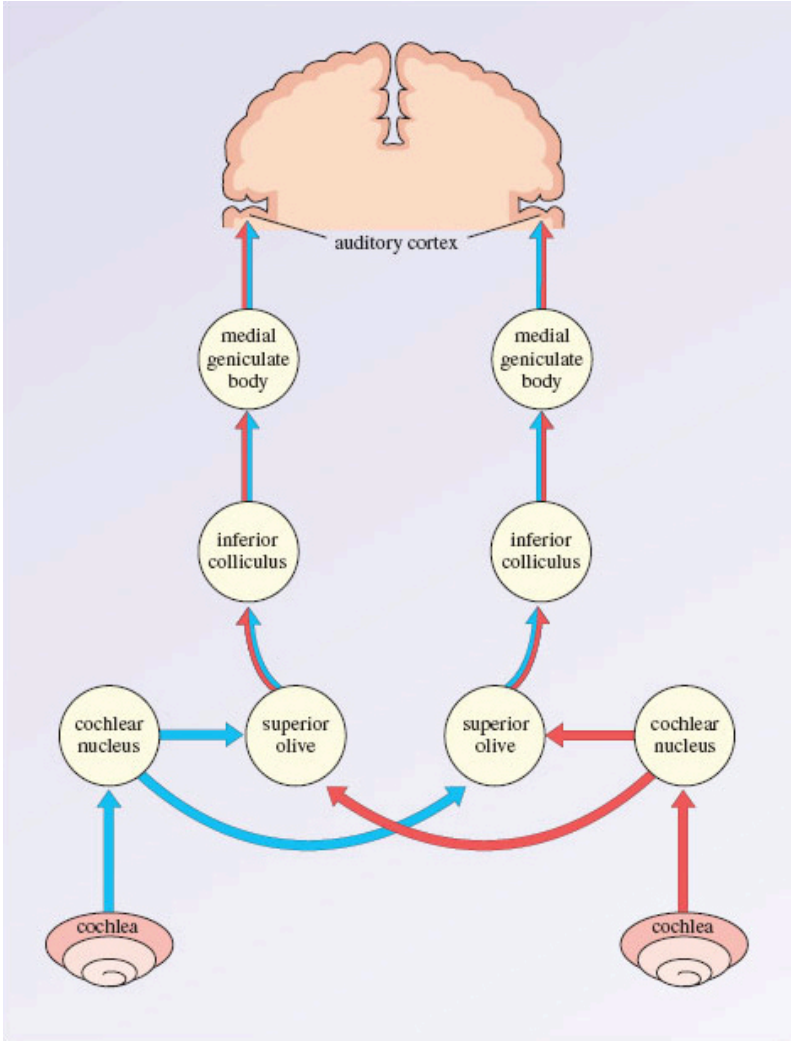


Figure 3. The ascending auditory pathway. Figure from The Open University.

Laboratory Methods

Auditory and Visual Reaction Times Methods

Set-Up:

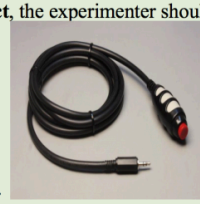
- Turn on the iWorx box.
- **Click** on the **Week 8 Settings file in the P-Drive**. (Click "this PC" and double click the Biol 256L Course Materials P Drive under "Network Locations). If you have trouble finding the file, it is also in the Week 08 Materials Link cybox folder.
- **Save** this file to your Desktop Folder.

Exercise 1: Reaction Time and Visual Cues

Aim: To **measure the reaction time** of a subject to a **VISUAL CUE**.

Notes for Experimenters in this Activity:

Out of sight of the subject, the experimenter should prepare to QUIETLY press and release the



button of the event marker

Warning:* *It is important to press and release the button of the event marker QUIETLY because any sound could be used by the subject as a cue.

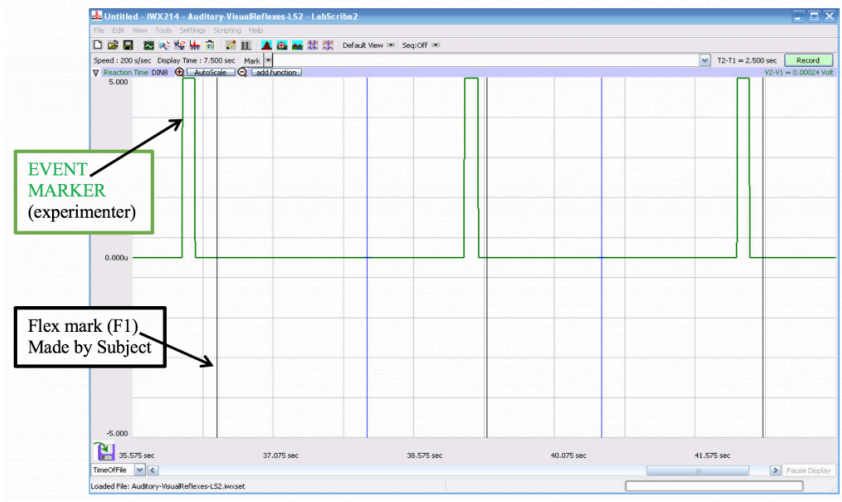
The experimenter should place the **STOP WATCH** next to one of the **Time Cue Cards (pink or blue),** in order to watch the stop watch run and also see the times on the cue card. Once the **RECORD** button is clicked, **START the STOP WATCH** and QUIETLY press the EVENT MARKER BUTTON at each indicated second on the cue card.

Procedure

1. **READ ALL METHODS** carefully before beginning to record.
2. Information for the **SUBJECT**:

- Instruct the subject to sit in a chair and face the computer screen.
- He or she should position a hand on the keyboard in a manner that enables the subject to push the **F1 key** as quickly as possible.
- Watch the **RIGHT SIDE** of the computer screen and quickly press the **F1** key on the keyboard when the signal generated by the event marker first appears.

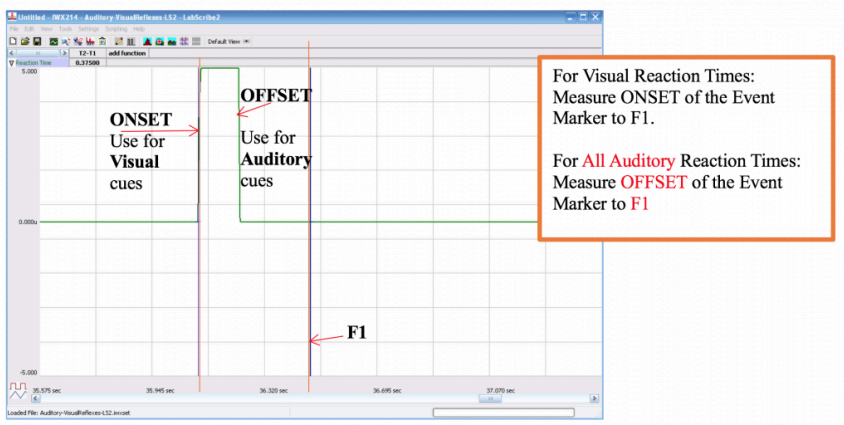
3. **Click** on the **RECORD** button.
4. **Type <Subject's Name> Visual Cues** in the **Mark box** to the right of the **Mark button**. Press the **Enter** key to mark the recording.
5. Instruct the subject to **press the F1 key** to mark the recording **as soon as he or she sees** the visual cue on the **Right** side of the computer screen (Methods Figure 1).
6. Instruct the subject that the exercise has begun and that a visual cue could appear on the screen at any time.
7. Use the event marker to deliver **ten** visual cues to the subject. The cues should not be **less than five seconds nor more than ten seconds apart**. Just follow the pink or blue **Time Cue Card using the provided timer.**
8. **After the tenth** cue, click **Stop** to halt recording.
9. Select **Save As** in the File menu, type a name for the file. Choose a destination on the computer in which to save the Data File, like your lab group folder. **Click** on the **Save button** to save the data file.



Methods Figure 1. *Three visual cues, each followed by the subject's response, are displayed on the Main window. Each visual cue is made by pushing the button of the EM-100 event marker momentarily; each response mark is made by the subject pushing the F1 key on the keyboard.* Three visual cues, each followed by the subject's response, are displayed on the Main window. Each visual cue is made by pushing the button of the EM-100 event marker momentarily; each response mark is made by the subject pushing the F1 key on the keyboard.

Data Analysis

1. **Scroll to the beginning** of the data recorded for **Exercise 1** to display the first trial on the Main window.
2. If you stay on the Main Window screen to gather data, look to the right-hand corner of the screen for **T2-T1=msec** for your time data.
3. Use the mouse to click on and drag a **cursor** to the **ONSET** of the **signal used as the visual cue**. Drag the other **cursor** over the mark made by the subject **(F1)**. See Methods Figure 2.
4. Once the cursors are placed in the correct positions for determining the reaction time, **record the value for T2-T1 on your Lab Report Worksheet.**
5. Once the reaction time in the first trial is measured and recorded, move to the data from the **second trial**.
6. Use the same techniques used in Steps 2 through 4 to measure the reaction times from the other eight trials.
7. Once the reaction times in all ten trials have been measured and recorded, **discard the longest** and **shortest times** from the data set, and determine the **average of the eight** remaining reaction times. **Record the mean reaction time for this exercise in the table on your Lab Report.**



Methods Figure 2. A visual cue, followed by the subject's response, are displayed on the Analysis window. The two cursors are positioned at the beginning of the visual cue and on the mark for measurement of the subject's reaction time (T2-T1) in this trial.

Exercise 2: Reaction Time and Auditory Cues

Aim: To measure the reaction time of a subject to an AUDITORY CUE.

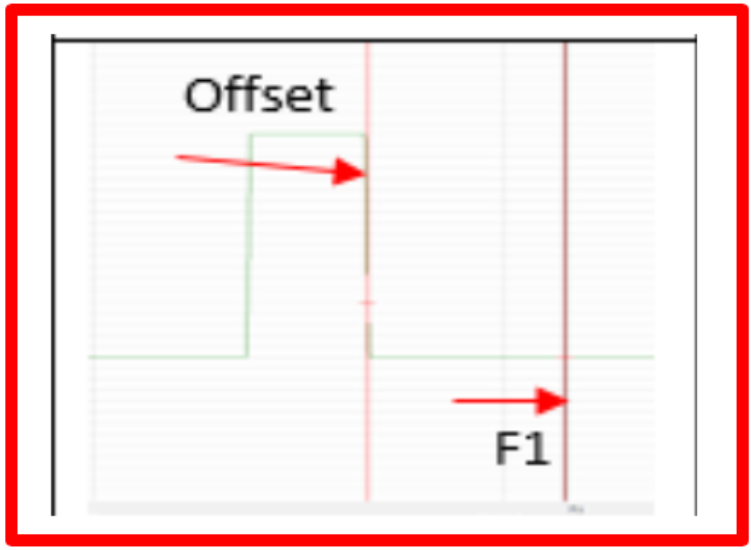
Procedure

1. Position the subject to **prevent her/him from seeing any signal on the screen** as a visual cue. Have the subject sit with their back to the screen and the computer key board within reach.
2. Instruct the subject to:

- Sit in a chair in front of the computer keyboard.
- Position a hand on the keyboard in a manner that enables the subject to push the **F1** key as quickly as possible.
- **Listen** for the **click (sound)** of the **event marker** as an experimenter presses the button and then **press the F1 key as quickly as possible**. (**Out of sight of the subject**, the experimenter taps the button of the event marker to create the auditory cue that is recorded as a signal on the Reaction Time channel. Make sure the student with the event marker in no way making contact with the student being tested.) In this exercise, the subject will perform **ten** trials.

3. **Click** on the **RECORD** button.
4. Type **Auditory Cues and Subject's Name in the Mark box** that is to the right of the Mark button and click **Enter.**
5. Instruct the subject that the exercise has begun and that an auditory cue could be heard at any time.
6. Use the event marker to **deliver ten auditory cues** to the subject. Follow the times on the **Time Card** (pink or blue, using the timer). The cues should not be less than five seconds nor more than ten seconds apart.
7. **Instruct the subject to press the F1 key** to mark the recording **as soon as he or she hears** the auditory cue made from the clicking of the event marker.
8. **After the tenth cue**, **Click Stop** to halt recording.
9. Select **SAVE** in the File menu.

Data Analysis



1. **Measure from the OFFSET to F1 to measure and record the reaction times** of the subject presented with auditory cues and all Auditory exercises from this point forward.
2. **Enter the mean reaction time** for this exercise in the table on your **Lab Report**.

Exercise 3: Reaction Time and Prompted Auditory Cues

Aim: To **measure the reaction time** of a subject to an **auditory cue delivered immediately AFTER a verbal prompt.**

Procedure

Repeat Exercise 2 with **THIS additional step**. **Before** each auditory cue is delivered, **tell the subject to GET READY** to respond to the cue. It is best to use a **one or two word cue** directly before clicking the event marker button.

Data Analysis

1. Use the same technique explained in **Exercise 2** to **measure and record the reaction times** of the subject presented with prompted auditory cues.
2. Enter the mean reaction time for this exercise in the table on your Lab Report.

Exercise 4: Reaction Time and Predictable Auditory Cues

Aim: To **measure the reaction time** of a subject to **auditory cues delivered at a predictable interval**.

Procedure

Repeat Exercise 2 with a predictable interval of **FIVE SECONDS** between each auditory cue. **Use the WHITE time card and the timer.**

Data Analysis

1. Use the **same technique explained in Exercise 2** to measure and record the reaction times of the subject presented with predictable auditory cues.
2. Enter the mean reaction time for this exercise in Table HN-1-L1 on your Lab report.

VOLUNTARY MUSCLE ACTIVITY

The Electromyogram

Karri Haen Whitmer

The movement of parts of the body is accomplished through a system of levers composed of skeletal muscles and bones. In a lever, the muscle attached to the bone provides the effort or force that moves the bone. As the muscle contracts and relaxes, flexes or extends, the bone rotates around a joint in the skeletal system. In relation to the muscles, the bones, and the body part being moved, the joint is the fixed point that functions as the fulcrum (point of rotation) for the lever. The body part being moved is the load on the lever. All levers, including the ones in the body, can be categorized into one of three classes, which are based on the position of the fulcrum in relation to the positions of the effort and the load (Figure 1).

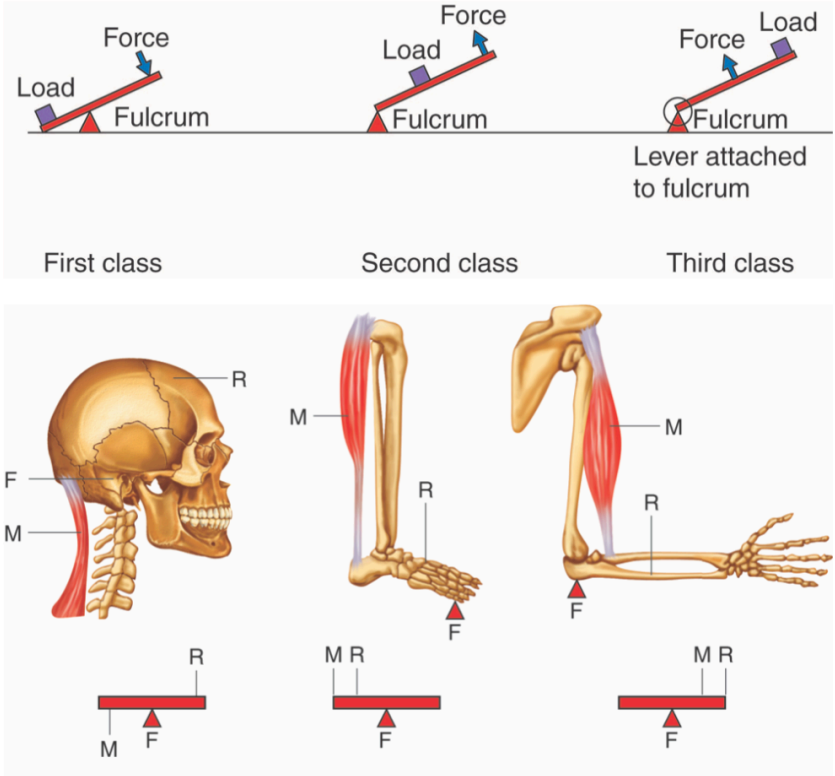


Figure 1:. Types of levers and their counterparts in the human body. The insertion of the muscle on the bone in the forearm places the effort between the elbow (fulcrum) and the hand (load). Image CC Attribution 4.0 License. Provided by OpenStax Anatomy & Physiology CC-BY-4.0.

Muscles are classified into functional groups depending upon how they contribute to joint movement. **Agonist** muscles are also referred to as "prime movers." The agonist provides the most force to complete the movement at a joint. **Antagonist** muscles are those whose action opposes the movement of the agonist. That is, typically when the agonist muscle contracts, the antagonist muscle must relax to allow movement. For example, during elbow flexion, the biceps are the agonist and the triceps are the antagonist. **Antagonistic muscle activity** can be seen as waxing and waning EMG trends when recording the biceps and triceps while flexing or extending the arm. Muscles classified as **synergists** stabilize a joint when it is moving. During elbow flexion, synergist muscles include the brachioradialis and brachialis: these assist the biceps and stabilize the elbow joint.

Voluntary Muscle Movement and EMG Activity

At this point in the course, we have created and analyzed several **electromyograms** (EMGs). This week, EMGs will record the electrical impulses from muscles during **sustained voluntary muscle contraction** during an arm wrestling match to help us understand how arm movement works. Unlike the smooth, wave-like EMGs seen for stretch reflexes and direct nerve stimulation, EMGs recorded during most voluntary muscle contractions are visualized as bursts of spike-like signals where the duration of the burst is proportional to the duration of the muscle activation. During voluntary muscle contraction, like we see in arm wrestling, *hundreds or thousands of motor units fire at the same time*. Additionally, the frequency of muscle fiber firing is greater in voluntary movement than in reflexes. Because of this, it becomes very difficult to quantify the amount of electrical activity in a muscle unless the **raw EMG data** is mathematically transformed. Most commonly, raw EMG data is transformed using the integration of the absolute values of the amplitudes of all the individual EMG spikes. This is called the **absolute integral**. *The area under the curve of the absolute integral of the EMG is linearly proportional to the strength of the voluntary muscle contraction*.

Increases in the force produced by a muscle are due to two physiological phenomena: recruitment and summation. During **recruitment**, increasing the stimulus amplitude on the muscle will result in more motor units responding to the stimulus, thus, increasing the tension in an entire muscle. During **summation**, muscle fibers become unable to relax due to increasing the stimulus frequency. Both phenomena result in increasing the force and duration of a muscle's contraction and contribute to sustaining strong muscular contractions over time.

Arm Wrestling Basics

The basic idea of arm wrestling is to pin an opponent's arm to the table. The general rules of arm wrestling are simple:

- Start the match sitting square to the table
- Keep both feet on the ground without bracing your feet or legs against any

solid object
- Elbows should be bent and against the wrestling surface, such as a lab table or a lab stool
- Grip opponent's hand
- Do not touch your body to your hand
- On the command "Start Wrestling" begin the Arm Wrestling Match
- Stay in the seated position throughout the entire match
- To win, a contestant must cause their opponent's hand or fingers to touch the table's surface
- Once the match is completed, Wrestlers should remain in the "Finished" position for measurement of the elbow joint angles

As the arm wrestling contestants begin the competition, muscle action potentials are generated to keep their muscles active and move their forearms to pin the opponent to the table. The muscles that are more active on an EMG will depend on whether the contestant is winning, gaining the advantage by pushing their opponent's arm to the table, or losing, being at a disadvantage when their own arm pushed toward the table.

Mechanics of an Arm Wrestling Match

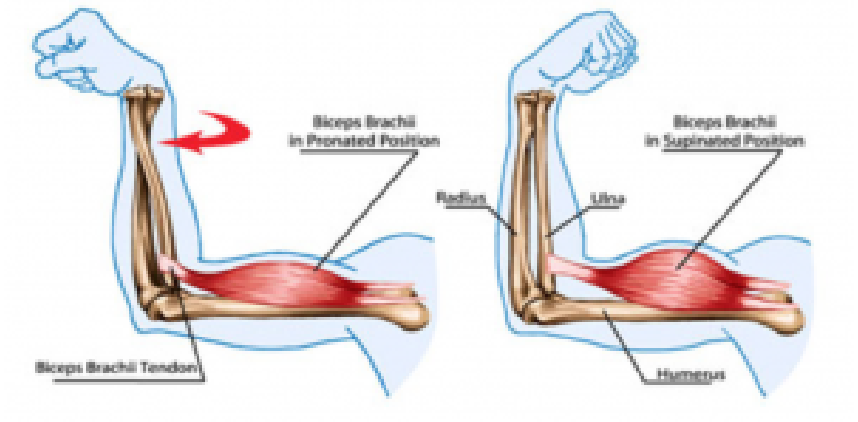
Arm wrestling involves the primary use of four muscles: the *Biceps brachii, Pronator teres, Pectoralis major* and *Flexor carpi ulnaris*. Other muscles such as the deltoid, *Latissimus dorsii* and *Triceps brachii* are also used. The forearm muscles are generally thought to be the most important with the upper arm and chest providing additional strength.

There are two factors involved in winning an arm wrestling match: *muscle fitness* and *arm wrestling technique.* In this lab, we will explore EMG responses to arm wrestling for two muscles: the *pronator teres* and the *biceps brachii* muscle. Using the EMG data, you may be able to differentiate whether technique or muscle mass was more important for the outcome of your matches.

Movements During a Match: Pronation and Supination

The ***pronator teres*** pronates the wrist and palm by crossing the ulna and radius. When you win an arm wrestling match, your wrist and palm will be completely pronated as you pin your opponent's hand to the table. When you lose an arm wrestling match, your wrist and palm will be completely supinated (the dorsum of the hand will be flat on the table in the "pinned" position). Once the arm is supinated, it becomes more difficult to push back into the neutral position.

The heads of the ***biceps brachii***, mostly known for flexing the elbow, exert force during supination and act as a accessory supinators. You can feel this if you put your hand on your biceps and pronate and supinate your palm with your elbow on the table: the biceps flex in the supinated position. During pronation and supination, the *biceps brachii* and *pronator teres* behave *antagonistically*.

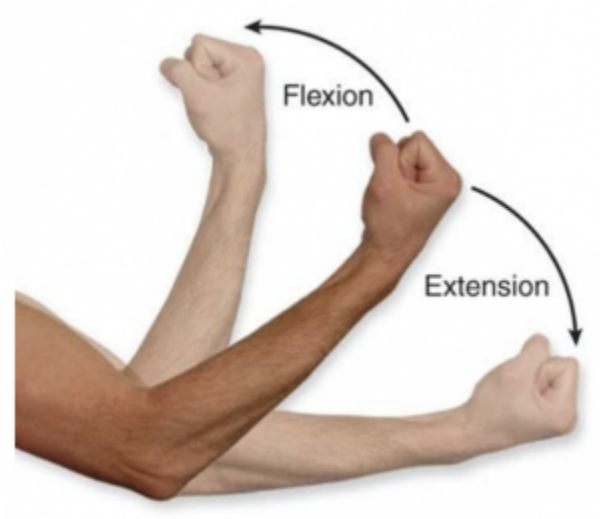


Movements During a Match: Elbow Flexion

Keeping the arm close to the body and the flexion of the elbow tight during arm wrestling is a commonly taught technique for helping to win a match. This is partially because the power of the **biceps brachii** muscle can be more easily harnessed to force the opponent's arm down onto the table in this position. Once the opponent's biceps are extended, they have to work much harder to win the match.

In the case of elbow flexion, both the pronator teres and the biceps brachii will act as elbow flexors under resistance; that is, they act *synergystically* to keep the elbow bent.

In general, **proper arm wrestling technique** involves keeping the arm at an acute angle and never allowing the arm to extend beyond 90 degrees. This posture allows the arm wrestler to more easily keep the arm close to the trunk and capitalizes on the strength of multiple muscles and ligaments. Once the opponent's arm is extended beyond 90 degrees, it will be very difficult for them to push back into a neutral position.



In this lab, you will compare the size of the upper and lower arm, EMGs and the angle of the humerus and radius/ulna to determine:

- Relative muscle exertion in the *pronator teres* and *biceps brachii* during resistance in an arm wrestling match.
- Whether muscle size, technique, or both lead to winning a match between two opponents.

Students participate in arm wrestling to observe and measure EMG activity while winning and losing. Electrodes will be placed on the *Biceps brachii* (upper arm) and *Pronator teres* (forearm) muscles to observe muscle action as different forces are used

to pin the opponent to the table. Detailed instructions of electrode placement are found in the Set-Up portion of this week's lab.

Laboratory Methods

- Record EMGs of voluntary muscle movement
- Record EMGs of voluntary muscle movement under stress

Start the Software

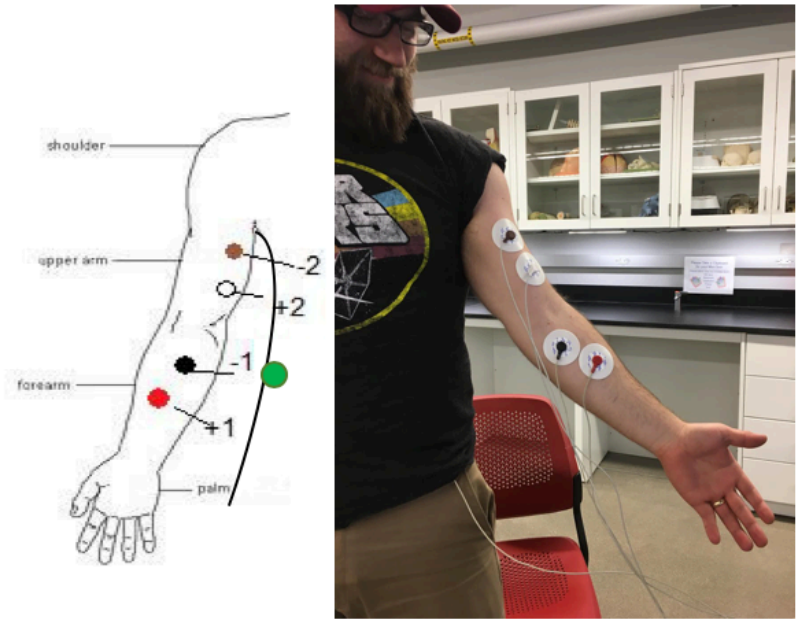
1. Turn on the iWorx unit.
2. Open the Week 9 settings file by clicking it in the P-Drive.

EMG Cable Setup

1.) Locate the muscles of the forearm and upper arm over which the recording electrodes will be placed. Muscles can be located by flexing or extending the hand and noting the areas of the forearm where the muscles are tense during these hand positions:

- One pair of recording electrodes will be placed over the Pronator teres muscle on the anterior surface of the forearm. The **pair of electrodes** should be placed side by side, with **2-4 centimeters** between them, on the length of the muscle as it crosses the inner forearm (Figure HM-7-S2). *Figure HM-7-S2: Location of Pronator teres muscle.*
- A **second pair of electrodes** will be placed over the **Biceps brachii** on the upper arm. The **first electrode in this pair** will be placed about **4 centimeters above the elbow**. The **second electrode in this pair** will be placed about **2-4 centimeters above the first, towards the shoulder.**

- **A fifth electrode**, used as **the ground**, is placed on the **lower abdomen**, **just above the waist,** on the **same side**.



Methods Figure 1. Electrode placement diagram and photo for voluntary arm movement. Note the ground electrode (green) on the inside of the shirt. Electrodes are placed on the dominant arm, only.

2.) Use an alcohol swab to clean and scrub the areas where the electrodes will be placed (Figure HM-7-S4). Let the areas dry before attaching the electrodes.

3.) Remove the plastic disk from a disposable electrode and apply it to one of the scrubbed areas. Attach an electrode to each of the other areas.

4.) Snap the recording lead wires onto the electrodes, so that:

- **THE RED** "+1" lead is attached to the electrode on the anterior forearm on the Pronator teres, close to the lateral edge of the arm towards the thumb.
- **THE BLACK** "-1" lead is attached to the electrode on the anterior forearm on the Pronator teres, closest to the middle of the forearm, near the elbow.
- **THE WHITE** "+2" lead is attached to the electrode on the anterior upper

arm on the Biceps brachii, near the elbow.

- **THE BROWN** "-2" lead is attached to the electrode on the anterior upper arm on the Biceps brachii, above the +2 electrode.
- **THE GREEN** "C" lead (the ground) is attached to the electrode on the lower abdomen.

Exercise 1: EMGs During Simulated Arm Wrestling

Aim: To study the EMG activity in muscles that work while simulating winning or losing during arm wrestling.

Note: Only one of the participants will have the electrodes attached to his/her arm at a time. Each student must participate as subject in Exercises 1 and 2. You may share data from the "between table" activity (Exercise 3).

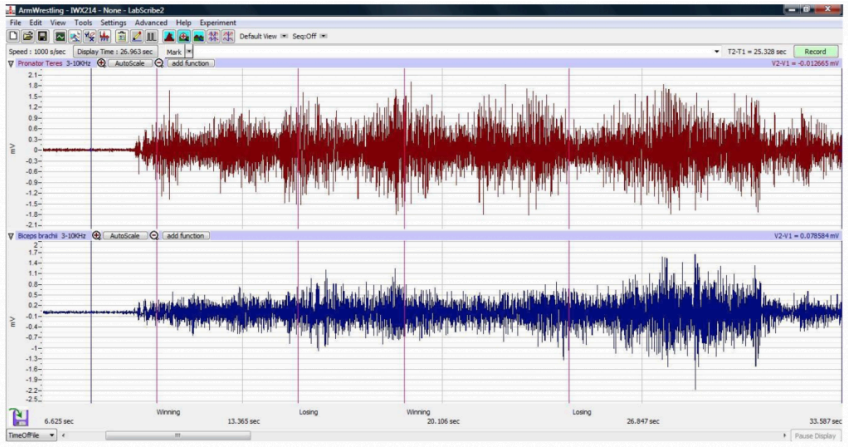
Procedure

1. **Click Record** and test the electrodes by:
    1. Making a tight fist and looking for corresponding EMG spikes on the upper Pronator teres screen
    2. Doing a bicep curl and checking for the bicep EMG on the lower Biceps brachii screen

2. **Type Neutral** in the **Mark box** to the right of the Mark button. **Click** on the **Mark button** to label the recording. Instruct the subject to place his or her arm in the **neutral position**, while clasping the hand of the opponent.
3. While the **subject's arm is in the neutral position**, type **Winning in the Mark box**. **When the subject pushes** his or her opponent's arm towards the table, **click** on the **Mark button** to label the recording.
4. While the subject's arm is in this position**, type Neutral in the Mark box**. When the **subject returns his or her arm to the neutral position**, **click** on the **Mark button** to label the recording.
5. While the **subject's arm is back in the neutral position**, **type Losing in the Mark box. When the subject's arm is pushed towards the table** by

the opponent, **click** on the **Mark** button to label the recording.

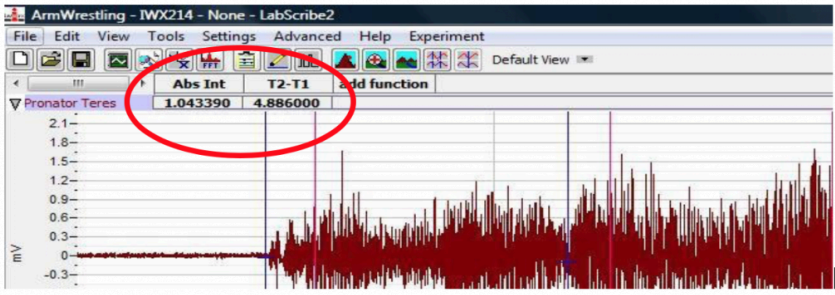
6. **Click** the **Stop** button.
7. Select **Save As** in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Designate the file type as *.iwxdata. Click on the Save button to save the data file.



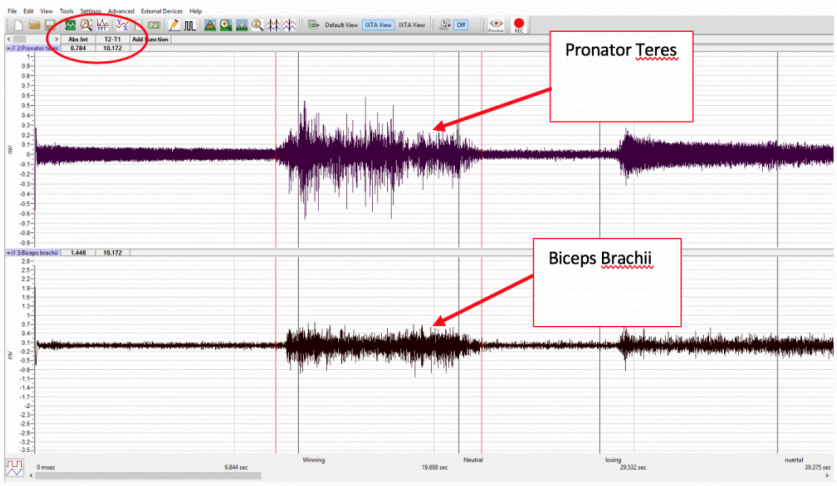
Methods Figure 2. Recordings from Pronator teres (top) and Biceps brachii (bottom) muscles of the arm during simulated arm wrestling.

Data Analysis

1. **Scroll** through the recording and find the section of data recorded while the subject was **simulating arm wrestling**.
2. **Click** on the **Analysis** window icon in the LabScribe toolbar.
3. Look at the Function Table. The **mathematical functions, Abs. Int. and T2-T1** should appear in this table.
4. **Absolute Integral** is the measurement of the total area under the EMG curve and is linearly proportional to strength of muscle contraction. **T2-T1** is the time measured in the same region as Abs. Int. in this experiment.



5. Use the mouse to **click** and drag the **double cursors** to the **onset** and **offset** of the **WINNING EMG burst** (including both the pronator and biceps EMGs) during the first "winning" cycle (Methods figure 3).



Methods Figure 3. Cursors are placed to measure the EMG activity taking place simultaneously in both muscles while "winning" at arm wrestling. Note – only one "win lose" cycle is shown.

6. **Record T2-T1 and the Abs Int values for each muscle** on your data table.

7. Use the mouse to move the cursors to onset and offset of the **LOSING EMG burst** (while the subject was "losing" at arm wrestling). Record the values for Abs. Int and T2-T1 from both muscles while "losing".

Exercise 2: EMGs During an Arm Wrestling Match

Aim: To study the EMG activity in muscles that work while winning or losing during an actual arm wrestling match.

Only one of the participants will have the electrodes attached to his/her arm at a time.

What are the rules for Arm Wrestling?

- Start square to the table.
- Keep both feet on the ground with out bracing your foot or legs against any solid object.
- Elbows should be bent and against the wrestling surface (table).
- Grip opponent's hand.
- Do not touch your body to your hand.
- On the command "Start Wrestling" begin the Arm Wrestling Match.
- Stay in the seated position throughout the entire match.
- Once the match is completed, Wrestlers should remain in the "Finished" position for measurement of the angles of the arms in both the Winner and Loser

Note: at the end of the match, ask opponents to hold their relative winning/losing positions in order to measure the arm angle with the goniometer.

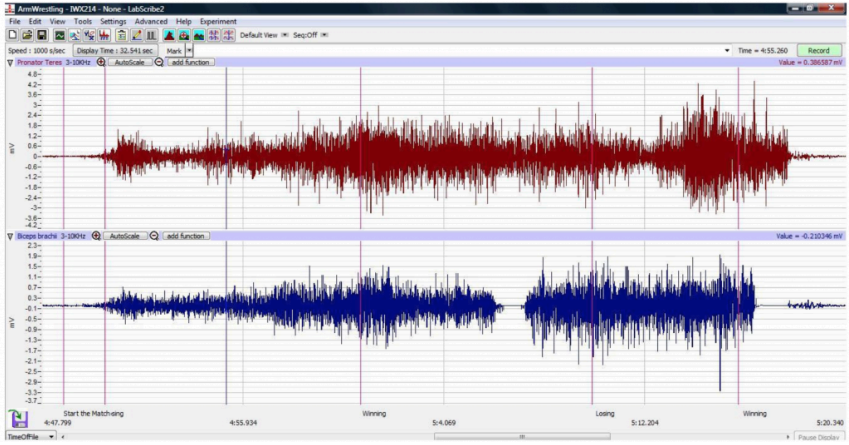
To start: record the circumference of the upper arm (biceps) and forearm (pronator) for each opponent by using a tape measure and wrapping it around the broadest area of the arm.

Procedure

1. Use the same experimental setup used in Exercise 1.
2. Instruct the subject to rest his or her forearm, with the electrodes, on a flat surface.
3. Have the participants clasp hands and get ready to arm wrestle.

Note: The person operating the computer needs to know when the subject's arm moves from a winning position to a losing position and back again. One student should act as the "spotter" and call out "Winning or Losing." The computer operator can simply type in W or L to indicate. This can occur relatively quickly, and some matches can be over in less than a minute.

4. **Click Record.** Instruct the subjects to begin arm wrestling at any time after the Record button has been clicked. Mark the recording "Start Wrestling".
5. Record the EMG activity from the muscles of the arm while the subject is winning or losing during the arm wrestling match. **Mark the recording appropriately when the subject's arm changes from a winning or losing position** (Methods Figure 4).
6. The match is over when the subject either pins his opponent's arm to the table or gets pinned by his/her opponent.
7. When the match is over, **click Stop** to halt the recording. **While the opponents remain in their winning/losing position, measure the angle made by the forearm and upper arm with the goniometer.**
8. Select Save in the File menu.



Methods Figure 4. Recording of an actual arm wrestling match between equally matched opponents.

Data Analysis

1. Scroll through the recording and find the section of data recorded while the subject was arm wrestling his/her opponent.
2. Use the same procedures used in Exercise 1 to measure and record the Abs. Int and T2- T1 from each muscle while winning or losing the match.
3. Record the values in **TABLES 1 and 2** of your Lab Report.
4. Select Save from the File menu.

Exercise 3: Winning Arm Wrestlers "Smack Down Match" Between Tables

Now a winning arm wrestler from each table will wrestle another table. Due to equipment upkeep concerns, **for this exercise, only one of the arm wrestlers from the two tables needs to be connected to electrodes. The two tables should share the data for the wrestler whose data is recorded.**

To start: record the circumference of the upper arm and forearm for each opponent by using a tape measure and wrapping it around the broadest area of the arm. When the match is over, be sure each opponent holds his/her winning or losing arm position to record the angle of the arms.

Use the same instructions from Exercise 2.

During the match, spotters should tell the computer operator whether the recorded opponent is Winning or Losing so it can be marked on the data.

- **Record the Winning and Losing EMG values** for one wrestler, and record arm sizes, and final elbow angles from both participants for this experiment (again, tables should share their data).
- Record data in Tables 1 and 3 (BETWEEN TABLES).
- Once the match is complete, measure the Abs. Int. and the T2-T1 for both muscles and report this data in the Lab Report.

ASSESSING CARDIOVASCULAR FUNCTION

The Electrocardiogram

Karri Haen Whitmer

Introduction to the cardiac cycle

Several physical and electrical events occur during the **cardiac cycle**, resulting in the flow of blood through the heart and peripheral tissues. In the simplest terms, the cardiac cycle traces the flow of blood through the heart. The physical contraction and relaxation of atria and ventricles is induced by the proper functioning of the heart's **electrical conduction system**.

At the beginning of the cardiac cycle, the atria are relaxed and fill with blood coming from the vena cavae and cardiac venus sinus or the pulmonary veins. The increased pressure in the atria due to atrial filling eventually forces the **atrioventricular (AV) valves** open, and blood flows into the ventricles. The atria contract and force the remaining blood into the ventricles, which are relaxed during filling.

As the pressure of the blood inside the ventricles increases, the atrioventricular valves close. During this time the arterial pressure is at its lowest point during the cardiac cycle; this is called **diastolic pressure.** Ventricular contraction increases the ventricular pressure until it exceeds the arterial pressure, forcing the **semilunar valves** open, causing blood to flow into the pulmonary arteries and the aorta. Blood entering the arterial system from the aorta inflates the arteries and increases blood pressure to a maximum, which is the **systolic pressure**.

In this lab you will record and **electrocardiogram (ECG)** from a subject and listen to the characteristic "lub-dub" heart sounds. The "lub" sound occurs during the early phase of ventricular contraction and is produced by closing of the atrioventricular valves, which prevents blood backflow into the atria. When the

ventricles relax, the blood pressure drops below what is in the arteries and the semilunar valves close, producing the "dub" sound. These sounds can be correlated with the basic waveforms seen on an ECG (Figure 1).

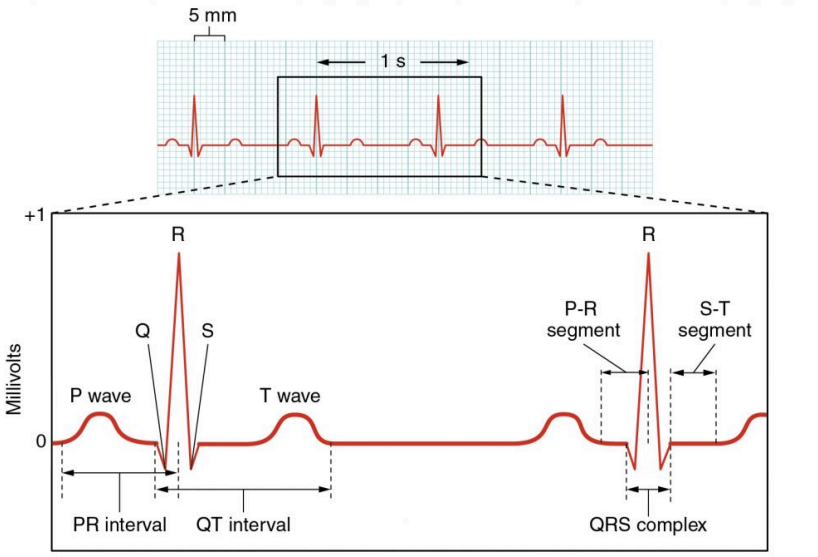


Figure 1. The standard ECG waveforms, intervals, and segments. A normal tracing shows the P wave, QRS complex, and T wave. Also indicated are the PR, QT, QRS, and ST intervals, plus the P-R and S-T segments. Figure from Open Stax Anatomy and Physiology CC BY 4.0.

Electrical conduction system of the heart

The heart is composed of two basic types of cells – contractile and non-contractile. Most cardiomyocytes are contractile, and when they are stimulated by an action potential, they contract. Some cardiomyocytes are non-contractile: that is, they only conduct action potentials to the contractile cells. These specialized cardiomyocytes are called **conducting fibers** (Figure 2).

Some of the conducting cells in the heart normally generate action potentials on their own (without nervous system stimulation). These cells, which lead to the **autorhythmic nature of the heart**, control the timing of contraction in the atria and ventricles. The most important autorhythmic cells of the heart are found in the **sinoatrial (SA) node** and the **atrioventricular (AV) node**. The **pacemaker** of the

heart, which sets the overall heart rhythm in normal individuals, is the SA node. The SA node generates and transmits signals, stimulating the atria to contract and sending an impulse to the AV node.

The signal sent from the SA node to the AV node informs the AV node cells that the atria have contracted. The AV node delays transmission of a secondary impulse to the rest of the heart. This short delay, normally 0.12 seconds, is very important because it allows enough time for complete atrial contraction before the ventricles contract (that is, enough time for the ventricles to finish filling with atrial blood). This delay coordinates the successive contractions of the atria and ventricles.

From here, the AV node sends an impulse through the **Bundle of His**, which quickly branches into the **right and left interventricular bundle branches**. These branches send the impulse through the ventricular septum of the heart. Once the impulse reaches the **apex of the heart**, ventricular contraction ensues. The two bundle branches taper to produce the **Purkinje fibers**, which stimulate the myocardial cells composing the ventricular walls to contract. Purkinje fibers first conduct the impulse to contractile cardiomyocytes at the apex and then to the cardiomyocytes of the lateral walls of the heart. The result is that the ventricles contract from the bottom upward, starting contraction at the apex and ending contraction in the regions of the ventricles proximal to the atria. This method of contraction allows for adequate squeezing of the blood out of the ventricles and into their respective arteries (either the pulmonary artery or aorta), much like squeezing a tube of toothpaste from the bottom up in order to force out all the toothpaste.

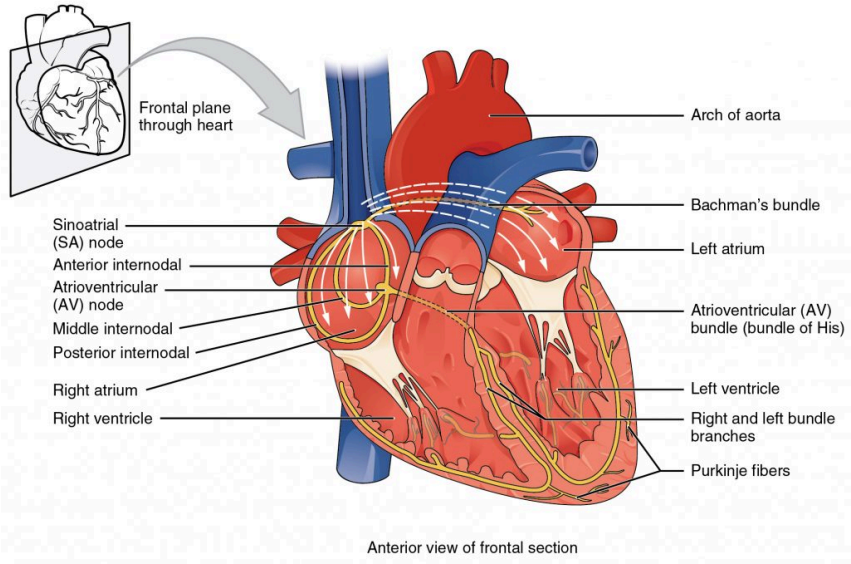


Figure 2. Electrical conduction system of the heart. Specialized conducting components of the heart include the sinoatrial node, the internodal pathways, the atrioventricular node, the atrioventricular bundle, the right and left bundle branches, and the Purkinje fibers. Figure from Open Stax Anatomy and Physiology CC BY 4.0.

Characteristics of the ECG

The electrical activity (action potentials) generated by the contractile cardiomyocytes of the heart during atrial and ventricular contraction can be measured by an **electrocardiogram (ECG)**. The most-simple ECG can be recorded by placing electrodes on the two wrists and left ankle, forming **Einthoven's Triangle**. ECG electrodes record the electrical potentials originating from the cells of the heart as impulses spread from the atria to the ventricles. These impulses cause very small electrical changes in the skin that occur in response to cardiac action potentials.

Although the timing and amplitudes for normal findings in the ECG may vary, the overall shape and direction (deflection) of ECG waveforms will be similar under normal physiological conditions (Figure 1, above). Combining ECG information with a patient's overall clinical status makes it an extremely valuable method for determining heart function. *Analysis of ECGs includes examination of ECG waves,*

intervals (a section of the ECG that contains a wave), and segments (an isoelectric, or flat, section of the ECG that is between two sequential waves).

The **P-wave** represents *depolarization of the plasma membranes* of **cardiomyocytes** leading to the sequential contraction of the right and left atria. Sometimes it is possible to see this sequential activation as a biphasic wave representing the spread of charge from the right to the left side of the heart. The duration of the P-wave is typically no greater than 0.12 seconds.

The **QRS complex** represents *depolarization* leading to the simultaneous contraction of cardiomyocytes of the right and left ventricles. Most of the electrical activity recorded by the QRS wave is derived from the left ventricle, which is larger and more muscular. The duration of the QRS complex is typically 0.06-0.10 seconds. The amplitude of the QRS complex may vary, depending on the size of the ventricles and the proximity of the electrodes to the ventricular chambers.

The **T-wave** represents the *repolarization* of the ventricles, which results in muscle cell relaxation.

Note: the *repolarization* wave of atrial cell membranes resulting in atrial relaxation is hidden within the QRS complex.

Measuring the time elapsed between ECG features can provide useful information when diagnosing heart abnormalities (Figure 3, and Table 1 below):

The **PR interval** is the region of the graph that extends from the beginning of the P wave (onset of atrial depolarization) until the start of the QRS complex (onset of ventricular depolarization). The duration of this interval is normally between .12-.20 seconds. The relatively slow conduction through this interval is important because it ensures that atrial and ventricular depolarizations are separated in time; thus, allowing adequate time for ventricular filling before the ventricles contract.

Pathologies of the Conduction System of the Heart:

Heart Block

Heart block refers to the conduction of impulses between the atria and ventricles that is too slow or non-existent. Long PR intervals can indicate **first degree heart block**, which is caused by conduction through the AV node that is too slow. Slow conduction through the AV node is called **AV delay**. AV delay may be caused by damage to the AV node tissues due to myocardial infarction (heart attack).

When AV conduction is completely blocked, the ECG displays back-to-back P waves. When P waves are not followed by the QRS complex, the ventricles fail to contract, and this leads to reduced mean arterial pressure (MAP) that can cause dizziness and fainting (due to the drop in cerebral blood pressure). When the ECG trace displays an occasional extra P-wave, where, otherwise, impulses still travel through the AV node , **second degree heart block** is diagnosed.

In the most severe case, P waves and QRS complexes may become independently regulated by the heart's conducting tissues because impulses never pass through the AV node. This condition leads to the atria and ventricles having their own distinct sinus rhythm, called **third degree heart block**. In this case, the ECG may show several extra P waves before a QRS complex.

The **Q-T interval**, as its name suggests, measures the time from the start of the Q wave to the end of the T wave. This measures the time for depolarization and repolarization, or contraction and relaxation, of the heart's ventricles. The Q-T interval is dependent upon heart rate: a faster heart rate results in a shorter Q-T interval. Reported values for normal QT intervals in resting individuals are equal to or lesser than 0.43 seconds. Longer than average Q-T intervals *might indicate* ventricular arrhythmias, depending on the resting heart rate and patient activity.

The **R-R interval** is similar to the Q-T interval in that it is also dependent upon heart rate. The R-R interval is the latency between QRS complexes, or the time

between successive depolarizations of the ventricles. The R-R interval measures the periodicity of the heart beat: that is, it measures the regularity of the heart beat. The R-R interval can be used to calculate **heart rate**.

The normal R-R interval is between 0.6 seconds to 1 second, and correlates to an average resting heart rate of 60-100 bpm. Heart rates vary in adults depending upon age, weight, fitness and other factors. Normal heart rates range from 60-100 bpm, but in highly trained athletes, the rate may be lower (40-60 bpm). Heart rates lower than 60 bpm are considered **bradycardia,** or abnormally slow. Heart rates over 100 bpm are considered **tachycardia**, or abnormally fast.

For a normal heart rate, we consider the **conduction velocity** of the myocardial cells of the conduction system of the heart. Just as we measured the conduction velocity of the ulnar nerve in the Week 7 laboratory, myocardial conducting tissues have their own normal conduction velocities, even though they are not neural tissue. That is, the speed at which action potentials are propagated within the tissue from one site to the next can be calculated.

The units for conduction velocity are distance/time, or in terms of the propagation of action potentials, are typically calculated in meters/second (m/s).

The conduction velocity in the AV node is the slowest of all the myocardial tissues (0.01-0.05 m/s). compare this value from the AV node with the conduction velocities for other myocardial tissues: atrial and ventricular conduction velocities average 1 m/s and the Bundle of His and Purkinje fibers average 2-4 m/s.

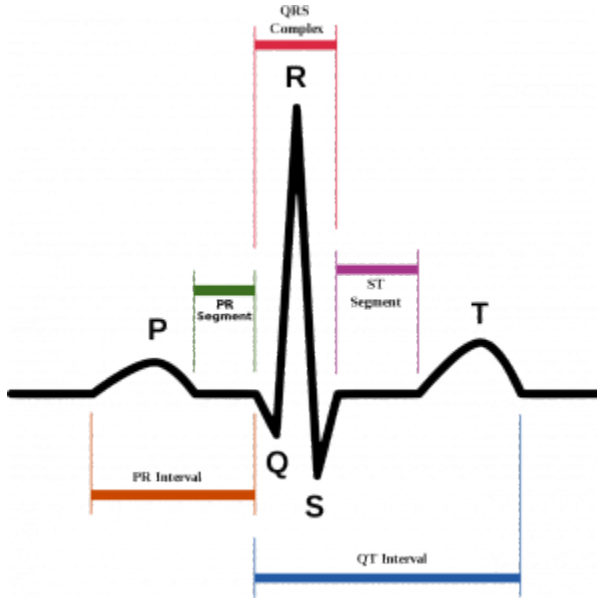


Figure 3. Typical time segments and intervals measured from ECG data during clinical evaluation.

Table 1. Major features of the ECG and their normal time values and descriptions.

ECG Feature	Duration (seconds)	Description
P Wave	.08-0.10 s	atrial depolarization
QRS Complex	.08-0.10 s	ventricular depolarization
T Wave	160 ms	ventricular repolarization
PR Interval	0.12-0.2 s	time between onset of atrial depolarization and ventricular depolarization
RR Interval	0.6-1.2 s	rate of ventricular cycle
PP Interval	0.6-1.2 s	rate of atrial cycle
QT Interval	.40-.43 s	time required for ventricular depolarization and repolarization
Heart Rate		60-100 BPM

Laboratory Methods

Conducting the Electrocardiogram and its Analysis

Equipment Required

iWire-B3G ECG cable and electrode lead wires, EM-220 Event marker, Stethoscope, Alcohol swabs, Disposable ECG electrodes

Start the Software

1. Turn on the iWorx unit with the switch at the back of the box.
2. Locate the P-drive and double click the Week 10 settings file to open LabScribe.

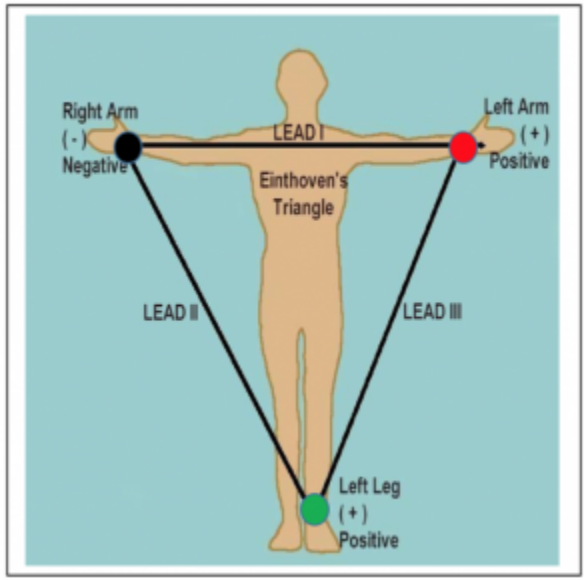
Set Up

1. Instruct the subject to **remove all jewelry from their wrists and ankles**. Use an alcohol swab to clean a region of skin on the inside of the subject's right wrist, left wrist and left ankle. Let the area dry.
2. Remove a disposable ECG electrode pad from its plastic shield and apply the electrode pad to the scrubbed area
3. Snap the lead wires onto the electrodes, so that:

- **The RED** (+1) lead is attached to the left wrist
- **THE BLACK** (-1) lead is connected to the right wrist,
- **THE GREEN** (C or ground) lead is connected to the left ankle.

4. Instruct the subject to sit quietly with their hands in their lap **holding the iWire B3G box**. If the subject moves, the ECG trace will move off the top or bottom of the screen. If the subject moves any muscles in the arms or upper body, electromyograms (EMGs) from the muscles will appear on the ECG recording as noise.

If in Exercise 1 you do not see a normal ECG recording, you may relocate the electrodes from Lead I to Lead II or Lead II (see figures 1a and 1b below).



Methods Figure 1. The ECG Lead I electrode configuration shown as Einthoven's Triangle.

Table 2. Electrode placement for Leads I, II and III. If the Lead I standard configuration does not produce the expected ECG waveforms, switch to Lead II or Lead III and reassess the ECG.

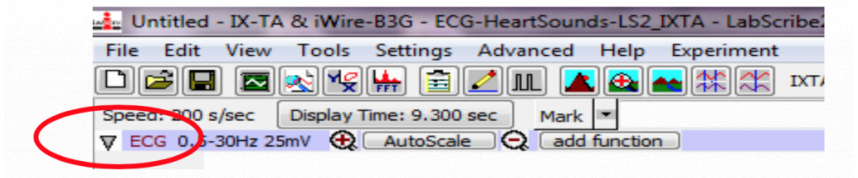
Lead	Red (positive)	Black (negative)	Green (ground)
I	**Left arm**, distal to the antecubital area	**Right arm**, distal to the antecubital area	Inside **left ankle**, behind the medial malleolus
II	Inside of **left ankle**, behind the medial malleolus	**Right arm**, distal to the antecubital area	Inside **right ankle**, behind the medial malleolus
III	Inside **left ankle**, behind the medial malleolus	**Left ar**m, distal to the antecubital area	Inside **right ankle**, behind the media malleolus

Exercise 1: The ECG in a Resting Subject

Aim: To measure the ECG in a resting individual.

Procedure

1. Click on the **Record button**, located on the upper right side of the LabScribe3 Main window. The signal should begin scrolling across the screen.
2. Click on the **AutoScale** button at the upper margin of the ECG channel. If the signal on the ECG channel is upside down compared to methods figure 2 (below), click on the **downward arrow** to the left of the channel title and **select the Invert** function. The trace should now look similar to the one in the figure.



3. When you have a suitable trace, **type <Subject's Name> Resting ECG in the Mark box** to the right of the Mark button. **Press the Enter** key on the keyboard to attach the comment to the data. **Record for a minute or two. Watch the time in the upper right-hand corner of the screen.**

4. **Click Stop** to halt recording.

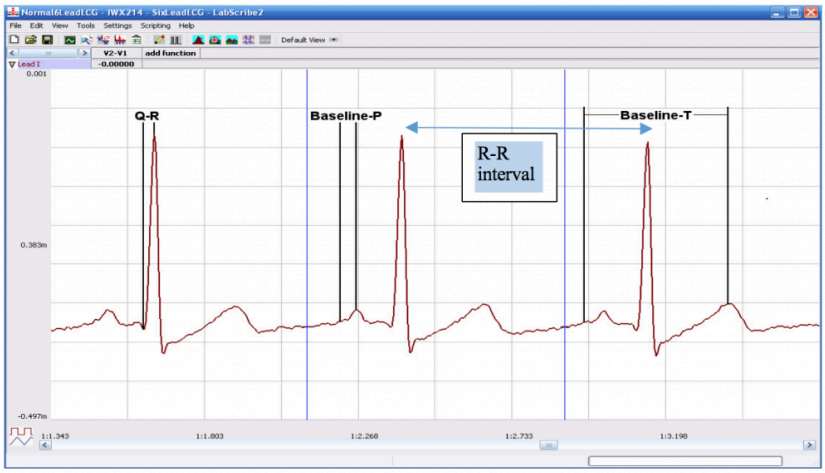
5. Select **Save As** in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder. Click on the Save button to save the data file.

Data Analysis

1. **Scroll** through the recording and **find a section of data with 4 to 6 optimal ECG cycles** in succession.

2. Use the mouse to click on and **drag the cursors** to specific points on the ECG recording to measure the following:

    - The **R-wave** amplitude. To measure the **R wave amplitude**, **place one cursor on the** Q **wave that precedes the R wave** and the **second cursor on the peak of the R wave**. The value for V2-V1 on the ECG channel is this amplitude. **Measure the amplitudes of two additional R waves** in **mV.**
    - The **P-wave** amplitude. To measure the **P wave amplitude**, **place one cursor on the baseline that precedes the P wave** and the **second cursor on the peak of the P wave**. The value for V2-V1 on the ECG channel is this amplitude. **Measure the amplitudes of two additional P waves** in **mV.**
    - The **T-wave** amplitude. To measure the **T wave amplitude**, **place one cursor on the baseline that precedes a P wave** and the **second cursor on the peak of the T wave** that is in **the same cycle as that P wave**. The value for V2-V1 on the ECG channel is this amplitude. **Measure the amplitudes of two additional T waves** in **mV**.
    - The **beat period**, which is the **time interval between two adjacent R waves (R-R interval)**. **To measure the beat period**, place **one cursor on the peak of a R wave and the second cursor on the peak of the adjacent R wave.** The value for **T2-T1** on the ECG channel is the beat period. **Measure the beat period for two additional pairs of R waves** in **seconds.**
    - Record the amplitude and time interval values in your lab report.



Methods figure 2. ECG recording. Lines and labels were added to indicate the locations where cursors should be placed to measure the amplitudes of R (Q-R), P (Baseline-P), and T (Baseline-T) waves and R-Wave to R-Wave (R-R interval).

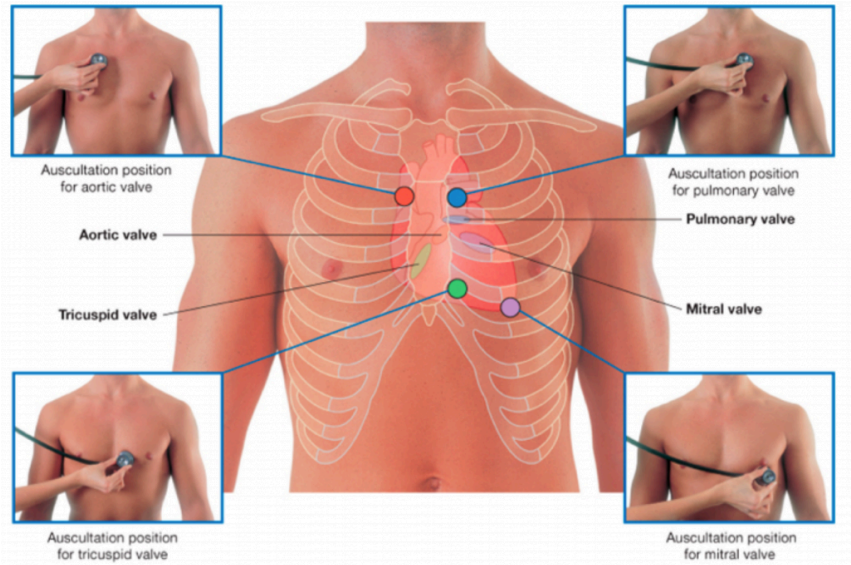
3. Calculate the following values and record on your lab report:

- The **average amplitudes for ECG waves and average beat period, in beats per second**.
- The **heart rate,** which is expressed in **beats per minute** and calculated from the average beat period by using the following equation:

Heart Rate (beats/minute) = (60 seconds/minute) / (# seconds/beat)

Exericse 2: The ECG and Heart Sounds

Aim: To study the relationship of heart sounds to the ECG.



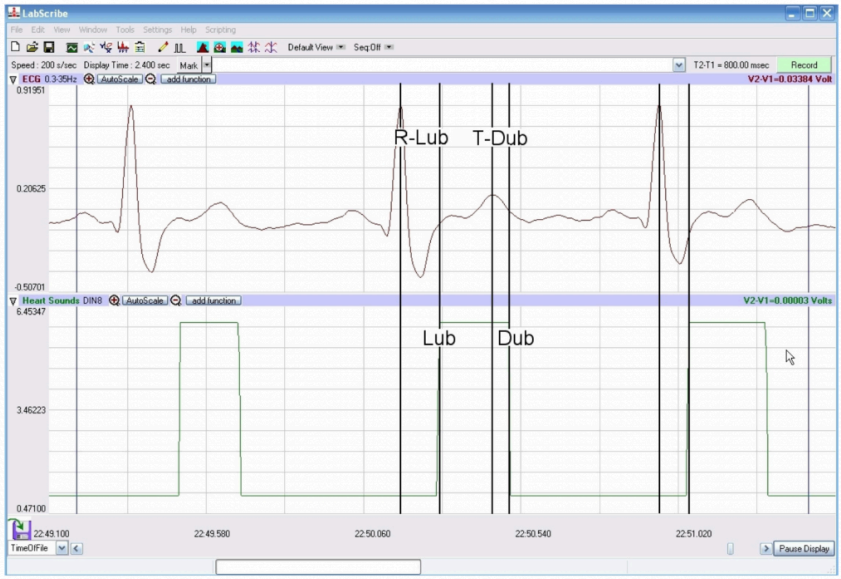
Methods figure 3. Auscultation positions for listening to the four heart valves.

Procedure

1. **Place the head of the stethoscope on the left side of the subject's chest** and **listen for the heart sounds**. Move the stethoscope head to the different auscultation positions shown above. Decide which position has the best-defined heart sounds.
2. **Click on the Record** button **(**you will record for **30 seconds)**. **With one hand, hold the stethoscope head** on the subject's chest where the "lub-dub" is heard the best, and hold the **event marker in the other hand. Press the event marker** when you hear the "**lub**", or first heart sound, and **release it when you hear the "dub**", or second heart sound. **This will happen very quickly! You should practice several times.**
3. **After recording for at least twenty seconds**, click **Stop** to halt recording. **After several tries, if the event marker for the "Lub" does not occur after the R-Wave and the event marker for the "Dub" does not align with or come right after the T-Wave, ask your TA for help.**
4. Select **Save** in the File menu on the LabScribe3 window.

Data Analysis

1. **Scroll** through the recording and find a section of data **with 4 to 6 exemplary ECG waveforms** and **consistent responses on the event marker channel, in succession.**



Methods figure 4. ECG and event marker recordings displayed in the Analysis window. Lines and labels were added to figure to indicate the locations where cursors should be placed to measure the time intervals between the R wave and the "lub" and the T wave and the "dub".

Use the mouse to click on and drag the cursors to specific points on the ECG recording to measure the following:

Note: Do the measurement for the R-Lub Interval and the T-Dub Interval on the same wave, making sure the event marker for the Lub is after the R-wave and the event marker for the Dub is after the T-Wave (Methods Figure 4).

1. The **R-Lub Interval**, which is the time **interval between the peak of a R wave and the onset of the event marker**. The onset of the event marker indicates the occurrence of the first heart sound or "lub". **Record** the value for

T2-T1 in **seconds.** Measure this time interval for **two additional ECG cycles**.

2. The **T-Dub Interval**, which is the **time interval between the peak of a T wave and the offset of the event marker.** The offset of the event marker indicates the occurrence of the second heart sound or "dub". **Record** the value for **T2-T1** in **seconds**. Measure this time interval for **two additional ECG cycles**.
3. Calculate the following values and record on your data table: **the average R-Lub interval in seconds; the average T-Dub interval in seconds**.

ASSESSMENT OF PULMONARY FUNCTION

Spirometry

Karri Haen Whitmer

Introduction

Over time, the amount of oxygen (O2) taken up and carbon dioxide (CO2) given off at the tissues is matched with the amount of O2 taken up and CO2 given off at the lungs. The exchange of O2 and CO2 across the respiratory membrane of the lungs relies upon diffusion between the air and the blood. Any change in the rate of diffusion could produce a change in breathing parameters. One factor that influences the rate of diffusion is pulmonary surface area, which may change due to injury or body position. All other parameters being held constant, an increase in the surface area will increase the rate of diffusion and thus decrease the rate and depth of breathing.

Respiratory controls and breathing rate

Changes in the body's metabolic demand also cause the respiratory control center in the medulla oblongata and pons to change the depth and rate of breathing to keep concentrations of blood gases, especially CO2 within homeostatic limits. The normal respiratory rate is 12-18 breaths per minute. Medullary control centers respond to elevated CO2 indirectly, by responding to a decline in pH as CO2 reacts with water. Then blood pH falls, the medullary control center stimulates an elevated breathing rate, to allow more CO2 to be expired. Once there is a normal pH detected, the breathing rate slows again to maintain a normal pH and CO2 concentration in your blood.

The control centers receive input from chemoreceptors in the carotid arteries and

aortic arch: the carotid bodies and aortic bodies. When these chemoreceptors detect elevated CO2, breathing is stimulated and the respiratory rate rises. When there is less CO2, the breathing rate falls. Low levels of blood O2 detected by aortic and carotid body cells can also increase the breathing rate.[1]

Pulmonary function test: Spirometry

Spirometry is a simple breathing test that measures how much air can be inhaled and evacuated from the lungs and how quickly air can be exhaled. During the spirometry procedure, a patient is asked to breathe into a mouthpiece while plugging the nose with a clip. The patient takes the largest breath possible and then blows it out as quickly as possible. The flow of air through the mouthpiece is detected by sensors that send information about flow stimulus to a computer. The graph of the volume of air expelled can be divided into different **lung volumes and capacities** (Figure 1).

1. Krumhardt, Ph.D.,M.T. (ASCP), B. (2013). Exercises in Human Physiology with Personal Health Assessment (Revised ed., p. 18). Redwood City, CA: Star Publishing Company, Inc.

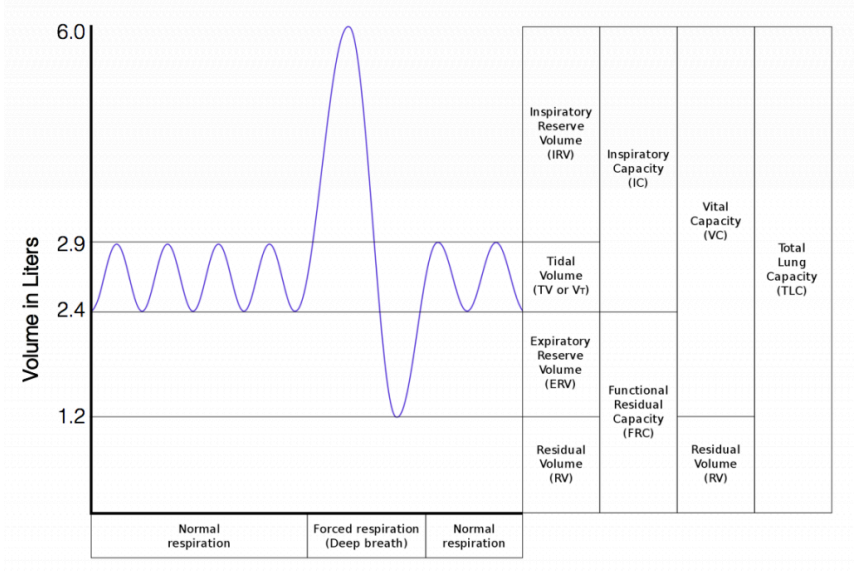


Figure 1. Graph of spirometry data for a normal male subject and corresponding lung volumes and capacities.

Respiratory Volumes and Capacities

Respiratory volume is the term used for various volumes of air moved by or associated with the lungs at a given point in the respiratory cycle. There are four major types of respiratory volumes: tidal, residual, inspiratory reserve, and expiratory reserve. Tidal volume (TV) is the amount of air that normally enters the lungs during quiet breathing, which is about 500 milliliters. Expiratory reserve volume (ERV) is the amount of air you can forcefully exhale past a normal tidal expiration, up to 1200 milliliters for men. Inspiratory reserve volume (IRV) is produced by a deep inhalation, past a tidal inspiration. This is the extra volume that can be brought into the lungs during a forced inspiration. Residual volume (RV) is the air left in the lungs if you exhale as much air as possible. The residual volume makes breathing easier by preventing the alveoli from collapsing. Respiratory volume is dependent on a variety of factors, and measuring the different types of respiratory volumes can provide important clues about a person's respiratory health.

Respiratory capacity is the combination of two or more selected volumes, which

further describes the amount of air in the lungs during a given time. For example, total lung capacity (TLC) is the sum of all of the lung volumes (TV, ERV, IRV, and RV), which represents the total amount of air a person can hold in the lungs after a forceful inhalation. TLC is about 6000 mL air for men, and about 4200 mL for women. Vital capacity (VC) is the amount of air a person can move into or out of his or her lungs, and is the sum of all of the volumes except residual volume (TV, ERV, and IRV), which is between 4000 and 5000 milliliters. Inspiratory capacity (IC) is the maximum amount of air that can be inhaled past a normal tidal expiration, is the sum of the tidal volume and inspiratory reserve volume. On the other hand, the functional residual capacity (FRC) is the amount of air that remains in the lung after a normal tidal expiration; it is the sum of expiratory reserve volume and residual volume.[2]

Comments on calculating values from the spirometry graph

Note that the spirometry graph records the variation in volume expelled through the spirometer flow head (usually represented in Liters) over time (in seconds). When reading the values for different spirometry measurements, it is important to use the correct Y-axis values. For example, to calculate the Tidal Volume from Figure 1, we subtract the lower limit of the measurement from the upper limit. E.g. TV = 2.9L-2.4L=0.5L. That is, the average value for TV in males is 0.5 L. Another example: the vital capacity in Figure 1 is 6.0L-1.2L=4.8 L. Note that a real spirometry graph in the doctor's office would not account for the residual volume (RV); thus, the low end value for vital capacity would be zero Liters. In our Figure 1, we include the residual volume as a teaching tool, so students can visualize all the values that account for the total lung capacity (TLC).

2. Betts et al. Open Stax Anatomy and Physiology. 2013. Section URL: https://openstax.org/books/anatomy-and-physiology/pages/22-3-the-process-of-breathing

Spirometry tells a clinician whether the lungs are functioning within the normal range. **Using the graph generated from spirometry, the clinician can make several measurements:**

- **Tidal volume (TV):** the volume of air displaced in the lungs during normal (tidal) inhalation and exhalation. This is the amount of air exchanged by breathing under normal, or resting, conditions. Note this value is a small percentage of the total lung capacity.
- **Inspiratory reserve volume (IRV):** the additional amount of air that can inflate the lungs after a tidal inhalation.
- **Inspiratory capacity (IC)**: the total amount of air that can be inhaled into the lungs (TV+IRV). A forceful inhalation can determine the IC with spirometry.
- **Expiratory reserve volume (ERV):** the amount of air that can be forcefully exhaled from lungs after a tidal exhalation.
- **Vital capacity (VC):** the maximum amount of air that can be expelled from the lungs after a maximal inhalation. VC = IRV + TV + ERV (see graph). The subject forcefully inhales and then forcefully exhales as much air as possible to determine the VC with spirometry. The vital capacity varies with height, gender and age of the subject.

Other important volumes or capacities that **cannot be determined directly by spirometry** include:

- **Residual volume (RV):** the amount of air left in the lungs after a forceful exhalation. This volume cannot be directly determined by spirometry because the amount cannot be expelled through the spirometer mouthpiece for measurement. This is the amount of air that always remains in the lungs, keeping the lungs from "collapsing."
- **Functional residual capacity (FRC)**: the amount of air remaining in the lungs after a tidal exhalation. FRC is unknown

in spirometry because the residual volume cannot be directly measured.

- **Total lung capacity (TLC):** the total air capacity of the lungs. TLC = VC + RV. TLC is unknown in spirometry because the residual volume cannot be directly measured.

Some additional measurements from the spirometry graph that are important for the clinical evaluation of lung function:

- **Forced vital capacity (FVC):** this is the total amount of air that can be forcefully expelled from fully inflated lungs during the spirometry test. FVC is equivalent to the vital capacity (VC) in most subjects.
- **Forced expiratory volume (FEV1):** the total amount of air blown out of fully inflated lungs after forceful exhalation for one second.
- **FEV1%:** the percentage of the FVC forcefully expelled from fully inflated lungs after one second. FEV1% = FEV1/FVC X 100%
- **Maximum inspiratory flow rate (MIFR):** maximum rate of air flow during inspiration.
- **Maximum expiratory flow rate (MERF):** maximum rate of air flow during exhalation.

Obstructive and Restrictive Pulmonary Disease

Pulmonary disease may be classified as either restrictive or obstructive, depending upon the cause of labored breathing. In a **restrictive lung disease**, the compliance, or elasticity, of the lung is reduced. When lung tissue becomes stiff or waterlogged, it cannot adequately expand. Common causes of decreased lung compliance are pulmonary fibrosis, pneumonia and pulmonary edema.

In **obstructive lung disease**, an obstruction results in increased airway resistance. For example, decreasing the diameter of an airway increases the resistance to airflow. Common obstructive diseases include asthma, bronchitis, and emphysema.

Spirometry as a Diagnostic Tool for Obstructive or Restrictive Disease

FVC and FEV values are critical measurements for diagnosing obstructive versus restrictive pulmonary disease. For example, in **obstructive pulmonary disease**, the FEV is diminished because it is difficult to force air out of the lungs due to airway narrowing. In some obstructive diseases like asthma, the residual volume is increased due to air trapping, resulting in a higher than normal FRC and TLC but a lower than normal FEV. In the case of **restrictive pulmonary disease** like pneumonia, the FVC, VC, TLC and FEV are reduced due to loss of elasticity of lung tissues.

FEV1/FVC (the FEV1 value divided by the FVC) is expressed as FEV1%. This ratio represents the percentage of the lung's vital capacity that a patient can expel in one second (Figure 2). Higher percentages are correlated with healthier lung tissue. Although the values for the predicted normal FEV1% will vary depending upon sex, age, and other demographics, a good rule of thumb is the FEV1% should be approximately 80%. In obstructive lung disease, the FEV1% is often much lower than normal, for example 40% as opposed to 80%, because it is very difficult to quickly force out air through a narrow airway (think of trying to forcefully exhale through a drinking straw). By contrast, in restrictive disease, the FEV1% may be normal (or high) because, although the vital capacity is reduced due to disease, the patient is still able to quickly force out a large percentage of the total expiratory volume into the spirometer.

Disease Type	FEV1%	FVC
Restrictive	80% or higher	low
Obstructive	Lower than 80%	normal or low

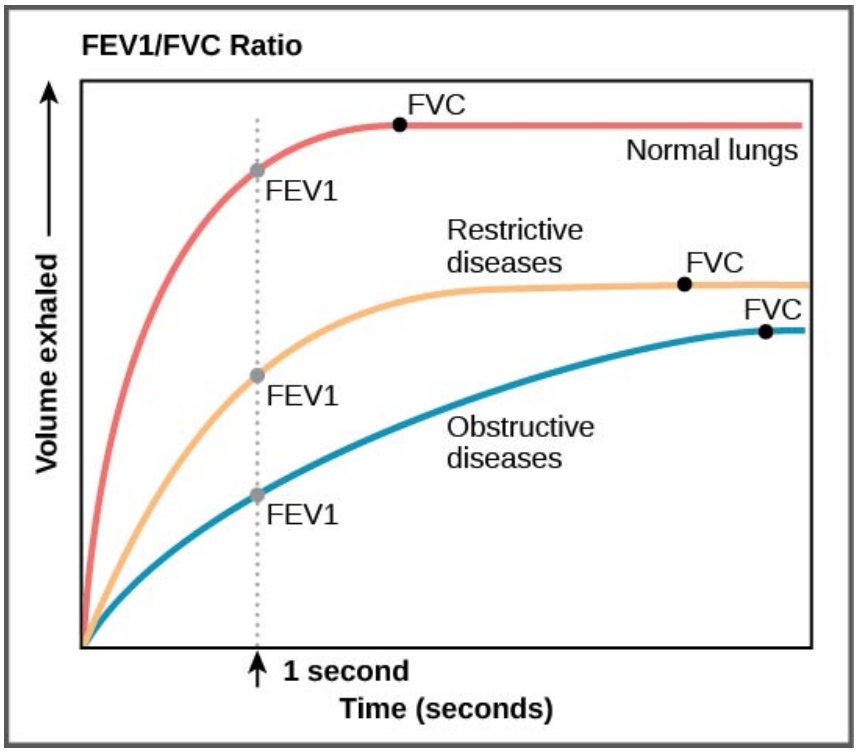


Figure 2. FEV1 and FCV for normal lungs, restrictive and obstructive pulmonary diseases. Most pulmonary disease results in reduced FEV1 and FVC values.

Body position, gravity and lung capacities

Various lung volumes and capacities will change when comparing the standing, sitting, and supine (lying down) positions. Inspiratory and expiratory volumes will be greater when sitting or standing than lying down. This is explained by gravity pulling the abdominal contents away from the diaphragm when upright, therefore increasing the volume of the thoracic cavity. In a supine position the patient's TV, IRV and ERV will decrease as the abdominal organs rest against the diaphragm limiting its movements. Thus, the vital capacity will decrease. The reduction in TV while laying down may partially explain why patients with respiratory disease have more difficulty breathing in the night while sleeping.

The volume of air passing into and out of the lungs in one minute is called **minute**

volume. This volume depends on both the depth of breathing (tidal volume) and the ventilation rate (breaths per minute). For example, minute volume increases during exercise and continues to be elevated for a short period after exercise. This is due to a combination of the increase in both rate and depth of breathing.

To calculate the minute volume: MV=TV*Rf, or, the minute volume is equal to tidal volume multiplied by the respiratory frequency (number of breaths per minute).

The minute volume influences but is not equal to the volume of air available for gas exchange in the alveoli, called the **alveolar ventilation**. Alveolar ventilation is less than the minute volume because the last part of each inspiration (and expiration) remains in the conducting passageways of the respiratory system. The part of the respiratory system where gas exchange between air and blood does not take place is called the **dead space**. **Anatomic dead space**, which generally averages 150 mL, is formed by the nasal cavity, pharynx, larynx, trachea, bronchi, bronchioles, and terminal bronchioles. **Physiological dead space** consists of anatomical dead space plus the volume of any alveoli in which gas exchange is less than normal.

In this clinical assessment, students will examine the effects of gravity on breathing by measuring the differences in lung volumes and capacities of a resting subject while he or she is sitting, standing, or lying down.

Laboratory Methods

Set-Up for Breathing and Gravity

Equipment Required: PC or Mac Computer, IXTA data acquisition unit, USB cable, Nose Clip, IXTA power supply, A-FH-300L Spirometer flow head and plastic tubes, Personal Blue Spirometry Flow Head

IXTA Setup Start the Software

1. Click on the LabScribe shortcut on the computer's desktop to open the program.
2. Select the settings file in the **Human Spirometry** folder then **Click** the **Breathing-Gravity-LS2**.

Spirometer Setup

1. Locate the A-FH-300L flow head and the airflow tubing (Methods Figure 1).

Methods Figure 1. The flow head connected to the internal spirometer of the IXTA unit. Blue personal Spirometry filter. Open neatly so it can be replaced and saved. Write your name and Table on bag.

Before Starting

1. **Please read the procedures for each exercise completely before beginning the experiment.** You should have a good understanding of how to perform these exercises before making recordings.
2. The spirometer will monitor breathing from a subject. It is important that the subject is healthy and has no history of serious respiratory or cardiovascular problems.
3. The **outlets on the flow head should always be in the upright position (Figure 1, left)** to avoid problems with condensation developing in the airflow tubes.
4. Attach the BLUE filter to the flow head on the side of the red marked outlet.
5. Turbulence in the flow head will produce a noisy signal. To reduce turbulence,

the subject should place his or her lips **COMPLETELY** around the outside of the opening of the BLUE bacterial filter attached to the flowhead.

6. Use a clip or pinch your nose with your fingers to prevent air from entering or leaving the nose as the subject is breathing. Air that passes through the nose is not included in the volume measurements and causes errors in these values.
7. The settings file**, Breathing-Gravity-LS2**, programs LabScribe to record the breathing of the subject on the Air Flow channel. A computed function is programmed on the Lung Volumes channel to convert the data recorded on the Air Flow channel to lung volume measurement.
8. **Allow the IXTA to warm up for 10 minutes** before recording for the first time.
9. When spirometry data is recorded in the conventional manner, inhalation is always displayed as an upward deflection. To determine if the subject is breathing through the correct end of the Blue flow head.

    - Click on the **Save to Disk button** in the **lower left corner** of the Main window to switch the LabScribe software into **Preview mode**. When LabScribe is in Preview mode, there is a **red X across the Save to Disk button**. In Preview mode, the iWorx **recording system works without recording data** on the hard drive or any other storage media which allows a subject to become comfortable with breathing through a spirometer.
    - **Click on the Preview button**. Have the subject inhale through the spirometer Blue flow head for **30-60 seconds**.
    - **Click on the AutoScale** button at the upper margin of the Air Flow and Lung Volumes channels.
    - If the White flow head is oriented properly, the traces on the Air Flow and Lung Volumes channels **will go UP during inhalation**.
    - If the traces on these channels **go down during inhalation, have the subject breathe through the other end of the Blue flow head.**

10. **Click on the Stop** button.
11. Before proceeding to the actual exercises, **make sure the LabScribe software in set to Record mode. Click on the Save to Disk button,** in the lower left corner of the Main window, to change LabScribe from Preview mode to

Record mode. When LabScribe is in Record mode, there is a **green arrow on the Save to Disk button.**

Exercise 1: Breathing While Sitting

Aim: To measure breathing parameters in a sitting subject.

Procedure

1. Instruct the subject to:

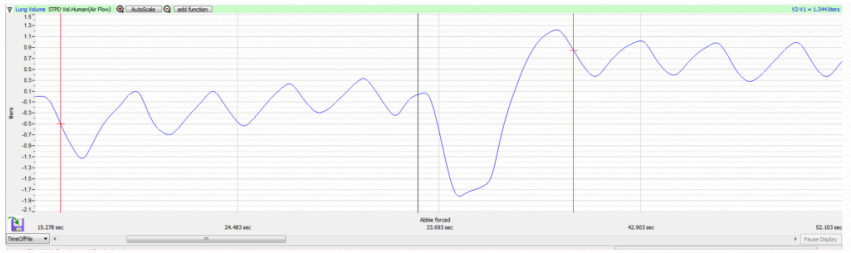
- Sit quietly and become accustomed to breathing through the spirometer Blue flow head as you did in the Set-Up Preview activity.
- Breathe normally before any recordings are made.
- Hold the White flow head so that its **outlets are pointed up (Methods Figure 1, left).**
- Remove the Blue and White flow heads from his or her mouth and hold it at mouth level in a position that prevents a breath from moving through either flow head.

Note: The LabScribe software will zero the Lung Volumes channel during the first ten seconds of recording. No air should be moving through the flow head during this time.

2. Type the Subject's Name/Sitting in the **Mark box** that is to the right of the Mark button.
3. **Click on the Record** button. After waiting **ten seconds** for the Lung Volumes channel to zero, have the **subject place the flowhead in his or her mouth and begin breathing**. **Press the Enter** key on the keyboard to mark the recording.
4. **Click the AutoScale** buttons of the **Lung Volumes channel**. Notice the slowly moving wave on the Lung Volumes channel. Record five breaths, which normally **takes about forty-five seconds to record.**
5. Type "**Forced"** in the Mark box. **Press the Enter key** on the keyboard **as the subject inhales as deeply as possible.** After **reaching his or her maximum**

inhalation volume, the subject should exhale as quickly and as completely as possible.

6. **After the forced exhalation is complete**, the subject should continue to **breathe normally through the spirometer for five breath cycles.**
7. **Click Stop** to halt recording. Your data may look like Methods Figure 2.
8. **Select Save** As in the File menu, type the subject's name (i.e. Alvin's Breathing and Gravity) 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.



Methods Figure 2. Air flow and lung volumes of the normal and forced breathing of a subject at rest.

Data Analysis-Normal Breathing While Sitting

1. Scroll through the recording and find the section of data recorded when the subject was breathing while resting, at the beginning of this exercise.
2. Use the **Display Time icons** to adjust the Display Time of the Main window to show at least four complete breathing cycles on the Main window. Four adjacent breathing cycles can also be selected by:

    - Placing the cursors on either side of a group of four complete breathing cycles; and
    - Clicking the **Zoom between Cursors** button on the LabScribe toolbar to expand the four selected breathing cycles to the width of the Main window.

3. Click on the **Analysis window icon** in the toolbar

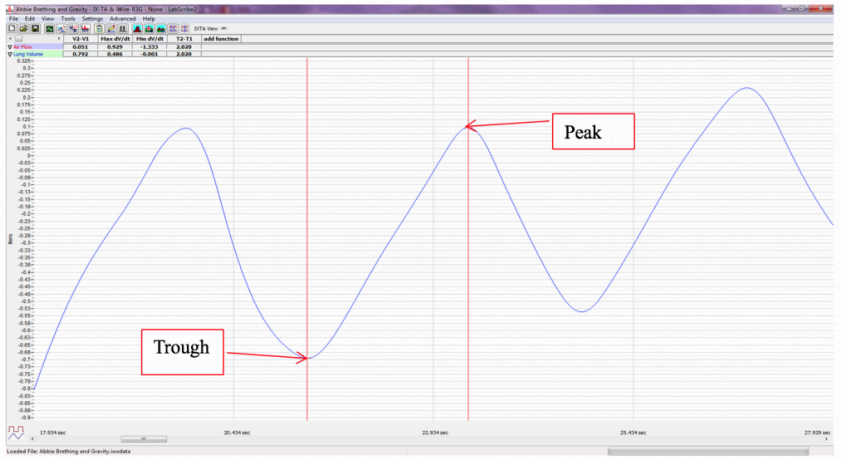
4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions, **V2-V1, Max_dv/dt, Min_dv/dt,** and **T2-T1** should appear in this table. Values for **V2-V1, Max_dv/dt, Min_dv/dt**, and **T2-T1** on each channel are seen in the table across the **top margin of each channel**.
5. **Maximize** the height of the trace on the **Lung Volumes channel** by clicking on the arrow to the left of the channel's title to open the channel menu. **Select Scale** from the menu and **AutoScale** from the Scale submenu to **increase the height** of the data on that channel.
6. Once the cursors are placed in the **correct positions** for determining the volumes and rates of each breath cycle, **as explained below** the values of the parameters in the Function Table can be recorded in your data table.
7. On the Lung Volumes channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following volumes:

    - **Tidal Volume (TV)**, which is the **volume of air inhaled or exhaled during a normal breathing cycle.** To measure the tidal volume of the subject during breathing at rest, place one cursor in the **trough prior to inhalation**, and the second cursor on the **peak** of the **cycle**. The value for the V2-V1 function on the Lung Volumes channel is the tidal volume **(Methods Figure 3). Measure and record 3 TV in Liters.**
    - **Maximum Inspiratory Flow Rate (MIFR)**, which is the **maximum rate of air movement during inhalation.** To **measure the maximum inspiratory flow rate** of the subject during breathing at rest, leave the cursors in the same positions used to measure the tidal volume **(Methods Figure 3).** The value for the **Max dv/dt function on the Lung Volumes channel** is the maximum inspiratory flow rate of that breath cycle**. Measure and record 3 in Liters.**
    - **Maximum Expiratory Flow Rate (MEFR),** which is the **maximum rate of air movement during exhalation. To measure** the maximum expiratory flow rate of the subject during breathing at rest, place one cursor on the peak of the breath cycle, and the second cursor in **the trough to the right of that peak**. The value for the Min_dv/dt function on the Lung Volumes channel is the maximum expiratory flow rate of that breath cycle **(Methods Figure 4)**.**This function is used**

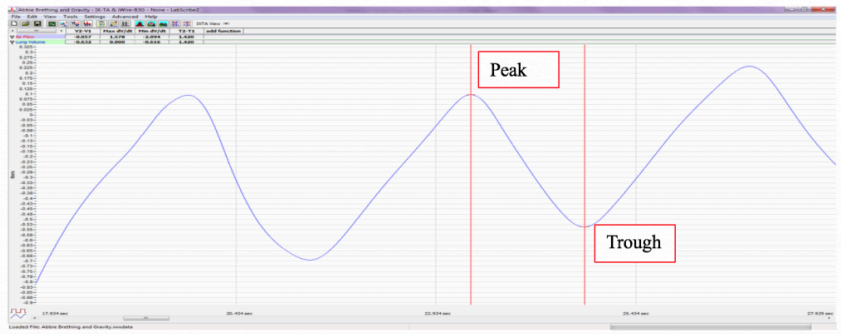
since the exhalation portion of the breath cycle has a negative slope. Measure and record 3 in Liters.

- **Breath Period**, which is the **duration of each breathing cycle.** To measure the breath period of the subject during breathing at rest, place one cursor on a **peak** of a **breath cycle**, and the second cursor on the **peak of an adjacent cycle**. The value for **T2-T1** on the Lung Volumes channel is the period of that breath cycle **(Methods Figure 5). Measure and record 3 Breath Periods in seconds.**

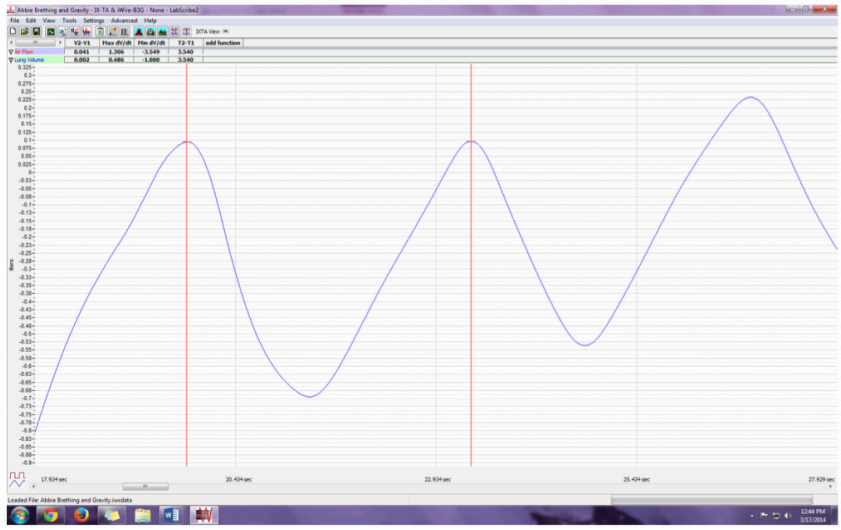
8. Record the values in your data table for Normal Breathing.
9. **Average the three values** obtained for each parameter and **enter the means in the table in your Data Table and Lab Report**.
10. Record the means for the tidal volume, rates, and breath period in the Data Table.
11. **Calculate the normal breathing rate** of the sitting subject using the following equation: **Breath Rate (breaths/minute) = 60 seconds/minute / mean breath period (sec/breath)**
12. **Multiply the mean tidal volume** by **the breathing rate** to **calculate the volume** of air passing in and out of the resting subject's lungs each minute.
13. Record the values for these calculations in the Data Table.



Methods Figure 3. Breathing pattern of a sitting subject displayed on the Lung Volumes channel in the Analysis window. The cursors are positioned on the trough and the peak of the breath cycle to measure the tidal volume (TV) with V2-V1 function and the maximum inspiratory flow rate with the Max_dv/dt function.



Methods Figure 4. Breathing pattern of a sitting subject displayed on the Lung Volumes channel in the Analysis window. The cursors are positioned on the peak of the breath cycle and the trough of the succeeding cycle to measure the maximum expiratory flow rate with the Min_dv/dt function.



Methods Figure 5. Breathing pattern of a sitting subject, displayed on the Lung Volumes channel in the Analysis window. The cursors are positioned on the peaks of successive breath cycles to measure the breath period with the T2-T1 function.

Data Analysis-Forced Expiration While Sitting

1. Use the slider or the arrows on the scroll bar, at the bottom of the Analysis window, to position the **Forced** expiration in the window.
2. Use the **Display Time icons** to adjust the Display Time of the Analysis window to show the forced expiration curve and the two normal breaths that occur before the forced expiration curve, on the same window. These breathing cycles can also be selected by:

    - Placing the cursors on **either side** of the group of appropriate breathing cycles; and
    - Clicking the **Zoom between Cursors** button on the LabScribe toolbar to expand the selected breathing cycles to the width of the Main window.The segment of the recording displayed in the window should be like the recording in **Methods Figure 6**.

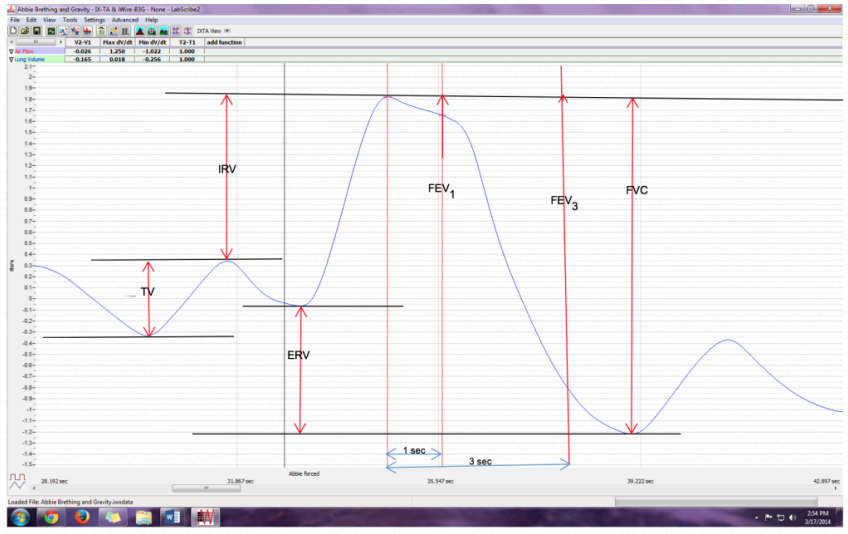
3. Place the cursors the forced expiration data on the **Lung Volumes channel** to measure the following volumes and rates using the **V2-V1**, **T2-T1**, **Max_dv/**

dt, and **Min_dv/dt** functions. Check the labels on **Methods Figure 6** to identify the volumes and rates that you will measure:

- **Tidal Volume (TV**), by placing one cursor in the **trough before the inhalation** segment of the resting breath and the second cursor on the **peak** of that resting breath cycle. The value for the V2-V1 function on the Lung Volumes channel is the tidal volume.
- **Inspiratory Reserve Volume (IRV),** by placing one cursor on the **peak** of the **normal breath prior to the maximum inhalation** and the second cursor on the **peak** of the **forced breath** cycle. The value for the **V2-V1** function on the Lung Volumes channel is the inspiratory reserve volume.
- **Forced Inspiratory Flow Rate**, by **keeping the cursors in the same positions** used for **measuring IRV**. The value for the **Max_dv/dt** function on the **Lung Volumes channel** is the **forced inspiratory flow rate**.
- **Forced Vital Capacity (FVC**), by placing one cursor on the **peak** of the **forced breath** cycle and the second cursor on the **flat line after the subject has expelled** all the air from his or her lungs. The value for the **V2-V1 function** on the **Lung Volumes** channel is the **forced vital capacity.**
- **Forced Expiratory Flow Rate,** by **keeping the cursors in the same positions** used for **measuring FVC**. The value for the **Min_dv/dt** function on the **Lung Volumes** channel is the **forced expiratory flow rate**.
- **Expiratory Reserve Volume (ERV)**, by placing one cursor in the **trough before maximal inhalation** and the second cursor on the **flat line after** subject has expelled all the air from his or her lungs. The value for the **V2-V1** function on the **Lung Volumes** channel is the **expiratory reserve volume**
- **Forced Expiratory Volume at 1 Second (FEV1)**, by placing one cursor on the **peak** of the **maximum breath cycle** and the second cursor on the **data point that is one second after the peak**. Use the **T2-T1** function **to determine** the data point that is **one second after the peak**. The value for the **V2-V1** function on the **Lung Volumes** channel is the

forced expiratory volume at one second.

- **Forced Expiratory Volume at 3 Seconds (FEV3),** by placing one cursor on the **peak** of the **maximum breath cycle** and the second cursor on the **data point that is three seconds after the peak**. Use the **T2-T1** function to **determine the data point** that is **three seconds after the peak**. The value for the **V2-V1 function** on the **Lung Volumes channel is the forced expiratory volume at three seconds.**



Methods Figure 6. Normal and forced lung volumes from a subject at rest, and displayed in the Analysis window. Lines and labels were added to figure to indicate to volumes that should be measured for each subject: Tidal Volume (TV), Inspiratory Reserve Volume (IRV), Expiratory Reserve Volume (ERV), Vital Capacity (VC), and Forced Expiratory Volume at 1 Second (FEV1).

4. Record these volumes and rates in the **Forced Expiration Table.**
5. Calculate the subject's FEV1/FVC ratio by dividing the subject's FEV1value by his or her FVC value.
6. Calculate the subject's FEV3/FVC ratio by dividing the subject's FEV3value by his or her FVC value.
7. Compare the FEV1/FVC and FEV3/FVC ratios of the subject to the normal values of 0.80 and 0.95, respectively, for young healthy adults. Both of these ratios decrease with age.

- In obstructive airway diseases, like asthma, bronchitis, or emphysema, both FVC and FEV1are reduced, and FEV1/FVC ratios are usually less than 0.70.
- In restrictive lung diseases, like fibrosis, FVC is reduced. But, because of the low compliance and high recoil of the lungs, the FEV1/FVC ratio may be normal (~0.80) or greater than normal (>0.85).

8. Record the FEV1/FVC and FEV3/FVC ratios in the table.

Exercise 2: Breathing While Standing

Aim: To measure various breathing parameters in a standing subject.

Procedure

Repeat Exercise 1 while the subject is standing.

Data Analysis

1. Use the same techniques used in Exercise 1 to measure the data recorded in Exercise 2. Record the measurements on your Data Table. Report the appropriate measurements in the tables.
2. Determine the values for the calculated parameters taken from the recordings of normal and forced breathing while standing. Report these values on the tables.

Exercise 3: Breathing While Supine

Aim: To measure various breathing parameters in a subject who is lying face up (supine).

Procedure

1. The subject should lie down on their back and relax.

2. Assist the subject when he or she is removing and replacing the flow Blue and white flow heads in his or her mouth. Place both of the flow heads on the bench top near the subject's head in a position that prevents any air to move through either of the flow heads.
3. Repeat Exercise 1 while the subject is supine.

Data Analysis

1. Perform the same types of measurements on the data recorded in Exercise 3 as were performed on the data recorded in Exercise 1. Record the measurements on your Data Table. Report the appropriate measurements in the tables.
2. Determine the values for the calculated parameters taken from the recordings of normal and forced breathing while supine. Report these values on the tables.

THE ELECTROOCULOGRAM

Karri Haen Whitmer

Eye movements can be recorded using electrodes placed on the skin near the eyes. This kind of recording is an electrooculogram or EOG. An EOG records eye movement because of a voltage difference between the cornea and the retina (Figure 1). As the eye moves, the vector of this electric field changes with respect to recording electrodes placed on the skin at fixed points.

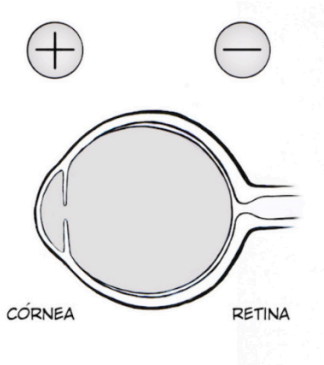


Figure 1. EOGs record eye movements due to the potential difference between the cornea and the retina.

Figure 1.

The human eye has six muscles attached to its exterior surface. These muscles are grouped into three antagonistic pairs that control horizontal, vertical, and torsional movement and position of the eye:

Horizontal axis:

- the medial rectus muscle turns the eye toward the nose (medially) (adducts)
- the lateral rectus muscle turns the eye away from nose (laterally) (abducts)

Vertical axis:

- the superior rectus muscle turns the eye up (elevates) with a slight rotation

toward the nose (medially) (adducts)
- the inferior rectus muscle turns the eye down (depresses) with a slight rotation towards the nose (medially) (adducts)

Torsional axis:

- the superior oblique muscle rotates the top of the eye toward the nose (laterally) (abducts) with a slight depression
- the inferior oblique muscle rotates the top of the eye away from the nose (laterally) (abducts) with a slight elevation

These muscles are innervated by motor neurons that have electrical activity with a tonic component that controls the position of the eye, and a phasic component that controls the velocity of eye movement. Even though the eye position commands and the eye velocity commands are linear functions of the firing frequency of the motor neuron, they are separate sets of commands. The eye velocity commands are sent along a direct path from specialized brain formations or fields to the motor neurons. However, the eye position commands are the products of the integration of eye velocity commands sent along an indirect path to a network of neurons that functions as a neural integrator. It is the output of the integrator that provides eye position commands to the motor neurons.

The integration of signals from different groups of neurons in the oculomotor system control five types of eye movement, each with a unique function and distinctive properties. These types are: saccades, pursuit, vestibular ocular reflex (VOR), vergence, and optokinetic reflex.

In this experiment, the subject will perform tasks that will generate electrical activity that will alter the standing voltage between the front and back of the eye that is correlated with horizontal eyeball movement. This movement will be obtained by electrodes placed on the skin near the eye. The record of this electrical activity is known as an electrooculogram (EOG).

These electrical changes are unique to each of four different types of eye movement (saccades, VOR, pursuit, and vergence). In our experiment, we will explore saccades, vestibular ocular reflex (VOR) and pursuit.

Saccades

The fovea centralis (focal point) is the region of the retina that sees in detail. Saccadic eye movements rotate both eyes so that image of interest falls on the fovea. You are using saccades at this very moment to point the fovea of your eyes at the words in this sentence. To compensate for the poor vision that occurs during saccades, saccadic movements are quick, with a velocity from 400 degrees to as high as 800 degrees of movement in a second.

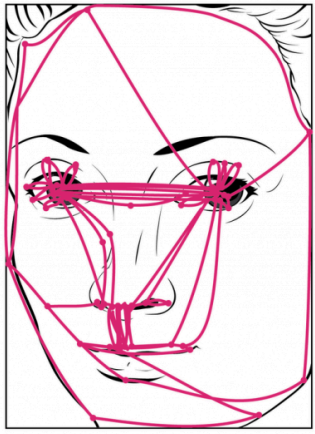


Figure 2. Saccades are rapid coordinated movements of the eyes to survey complicated visual stimuli. This image represents the shifts in gaze typical of a person studying a face. Notice the concentration of gaze on the major features of the face and the large number of paths traced between the eyes or around the mouth. Image CC Attribution 4.0 License. Provided by OpenStax Anatomy & Physiology CC-4.0

Figure 2. From OpenStax Anatomy and Physiology, licensed CC BY 4.0.

Saccades are also accurate because the saccadic system uses an internal estimate of eye position from its neural integrator to guide and stop the saccades. When looking at an object, saccades move the eye in all potential directions in the orbit, but they are interspersed with pauses in eye movement, called fixations (see the trace of eye movement in Figure 2). During fixations, the gaze is directed to a single location, only. In Figure 2, saccadic eye movement results in eye movements that allow the subject to view the different components of the picture, while fixations allow the subject to stop eye movement and focus on a single detailed feature of the picture.

Vestibular Ocular Reflex (VOR)

The vestibular ocular reflex keeps the image of the outside world stationary on the

entire retina when the head moves. VOR connects lateral movement of the head in one direction with lateral movement of the eyes in the opposite direction. For example, if you rotate your head to the right as you look at this word, your eyes rotate to the left. VOR is a phasic response that is faster than pursuit because it is a simple central reflex arc that involves only three neurons. The signal that indicates the velocity of head movement originates in the semicircular canals of the ear and goes through an afferent nerve and an interneuron on its way to the motor neurons of the oculomotor muscles. The muscles rotate the eyes at a velocity that matches the velocity of the head, thus, keeping the image stationary on the retina.

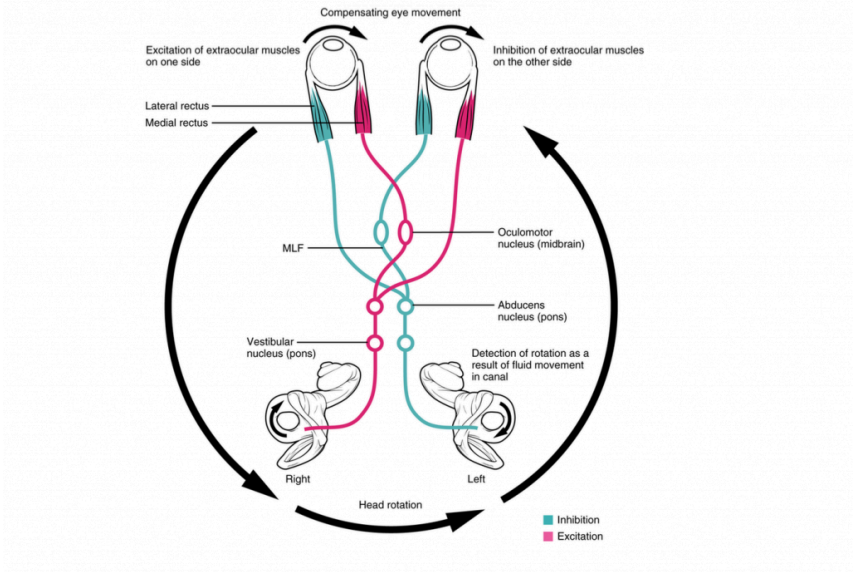


Figure 3. If the head is turned in one direction, the coordination of that movement with the fixation of the eyes on a visual stimulus involves a circuit that ties the vestibular sense with eye movement. Image CC Attribution 4.0 License. Provided by OpenStax Anatomy & Physiology CC-4.0

Figure 3. From OpenStax Anatomy and Physiology, licensed CC BY 4.0.

The eyes are held on the image through a tonic response along an indirect path through a reverberating neural circuit between the afferent nerve and motor neurons. Without this neural circuit, which is a short-term memory device, the eyes would drift back to center and off the image while the head was still rotated.

Smooth Pursuit Eye Movements

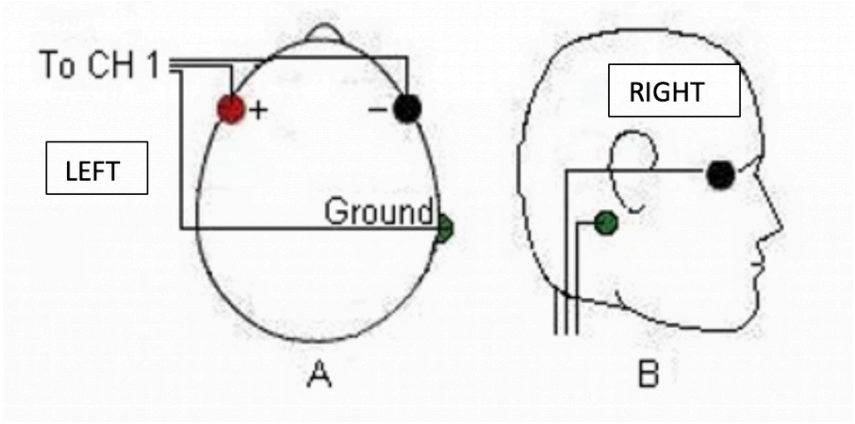
Pursuit eye movement keeps the fovea pointed at a moving target, like a ball rolling on the horizon. Smooth pursuit movements may be vertical or horizontal, and each will produce a different pattern on the EOG. There is an initial delay (latency) in pursuit movement because the signal from the eye, that indicates the object is moving, has to be conducted through many synapses to the brainstem. Initially, when the object starts to move, a saccade helps the fovea catch up to the object until the pursuit movement begins to track the object. Smooth pursuit movements continuously track the moving target through eye movement, only (no head movement). Smooth pursuit movements are slow (up to 30 degrees per minute) and do not require fixations for focusing.

Methods

Set up

Start the Software

1. Click on LabScribe3, Click Settings→ Human Muscle→ EOG
2. All Students will be a subject in this experiment. Select the first person from your group to be the subject in this experiment.
3. Use an alcohol swab to clean and scrub the areas where the electrodes will be placed (Methods Figure 1). Let the areas dry before attaching the electrodes.
4. Trim the electrodes and then remove the plastic disk. Apply the electrodes to one of the scrubbed areas. Attach an electrode to each of the other areas.
5. Attach the electrodes so that the red lead is located next to the left eye, the black lead is located next to the right eye, and the green ground is located under the right ear on/near the mastoid process.
6. Electrode pads can be cut to avoid the hairline lateral to each eye and the hair line in the mastoid process area.
7. Drape the leads for the electrodes and have the subject place the iWire electrode box on their lap. **There should be no tension on the electrodes.**
8. The subject should sit quietly with their hands and iWire box on their lap.



Set-up Figure 1. Diagram showing electrode position for the EOG.

Week 12: Electrooculogram Activity (EOG)

Note: for these activities, it is important to observe your subject's physical eye movements.

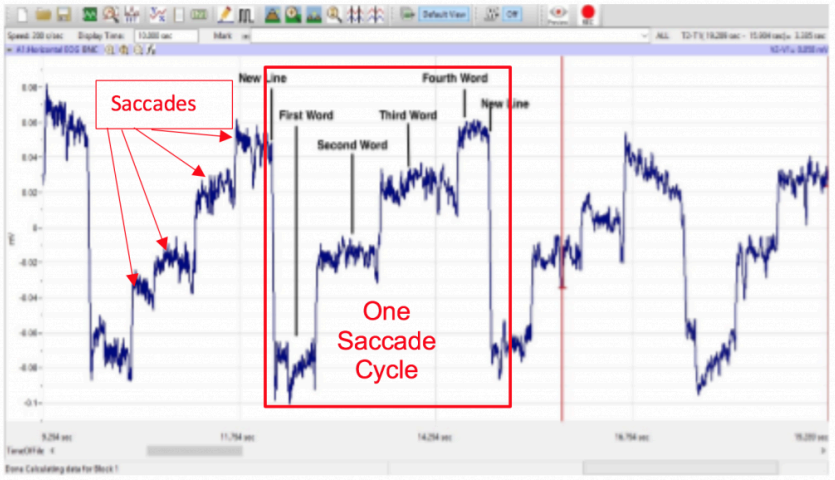
Exercise 1: Saccades

Aim: To demonstrate the type of electrical activity that occurs in the muscles as the subject is reading using paragraphs in different settings.

The number of words in each line, the length of each line, and the formatting of the paragraph will affect the shape of the EOG recording.

Control:

- Once the electrode pads and leads are placed
- Subject will **close their eyes** and move their eyes **left to right 4 times, and up and down 4 times and blink your eyes 4times** while being recorded. This will provide a baseline and proof that electrodes are in the correct positions.
- Type in Mark box subject's name, control, once you start Recording, click on Mark to place wording on data screen.



Methods Figure 1. Electrooculogram (EOG) of a subject reading slowly. Four to Six words are on each line. As the subject moves his eyes to the right to read the next word, the level of the recording rises a step. As the subject moves his eyes back to the beginning of the next line, the amplitude of the EOG drops. **Note: the trace may be inverted depending on electrode placement.**

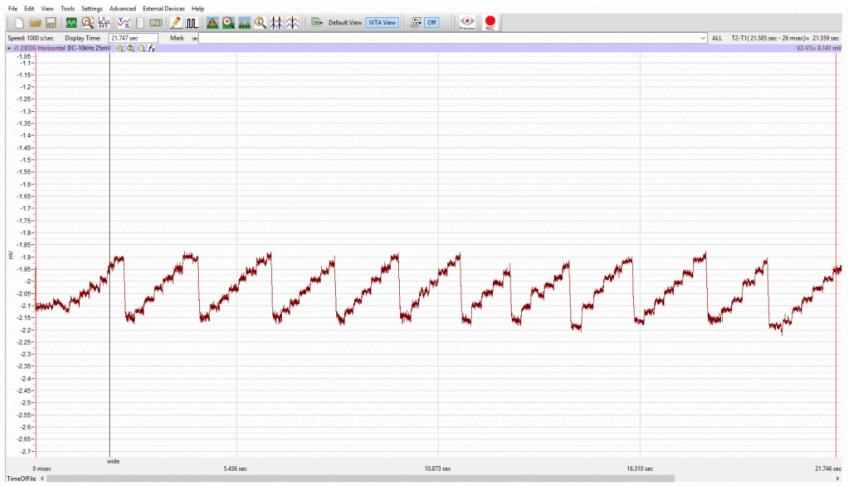
Narrow Normal Saccades

1. Procedure for **Narrow Normal Paragraph. Select writing sample #4 Narrow Normal Paragraph** for the subject to read as his or her EOG is recording.
2. The subject should avoid any voluntary movements of his or her head or body during the recording. ALL READING ARE TO BE DONE SILENTLY WITHOUT MOVING ANY FACIAL MUSCLES. Only the subject's eyes should be moving while he or she is reading. **Have subject hold the document at eye level.**
3. Type "**Narrow Normal Saccades**" in the **Mark** box to the right of the Mark button on the LabScribe3 Main window.
4. As the subject is **focusing on the first word** in the paragraph, **click Record**. Instruct the subject to begin reading as you **click on the Mark** button to mark the recording.
5. After the first cyclic pattern in completed, click on the AutoScale button. Observe the subject's eyes as he or she is reading.

6. **Click Stop** to halt recording when the subject has finished reading.
7. **Select Save** As in the File menu, type Narrow Normal Paragraph and the subject's name for the file. Click on the Save button to save the data file to Desktop to your Table's Folder if desired.

Wide Normal Saccades

1. Procedure for **Wide Normal Paragraph. Select writing sample #11.**
2. The subject should avoid any voluntary movements of his or her head or body during the recording. Only the subject's eyes should be moving while he or she is reading. **Have subject hold the document at eye level.**
3. Type "**Wide Normal Saccades**" in the **Mark** box to the right of the Mark button on the LabScribe3 Main window. As the subject is **focusing on the first word** in the paragraph, **click Record**. Click the mark button to mark the start of the reading.
4. Click stop and save the recording.



Methods Figure 2. Electrooculogram (EOG) recording the saccadic eye movement of a subject reading the Wide Normal Paragraph.

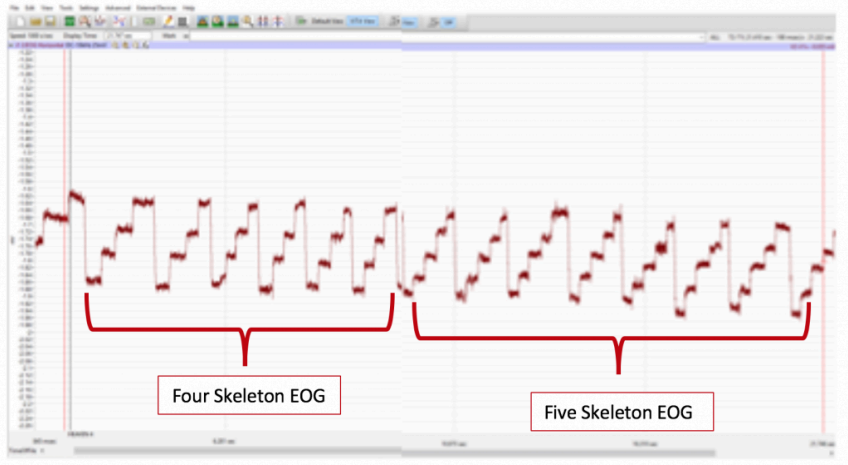
Wide Slow Saccades

1. **Type Wide Slow Saccades** in the **Mark box** to the right of the Mark button

on the LabScribe3 Main window.

2. Instruct the same subject: **hold the document at eye level and move from word to word** in the **same paragraph** using a **slower saccadic movement. FOCUS on each word FOR TWO SECONDS**, to do this **say the word to yourself twice before moving to the next word. (Example:** A,A, neurologist, neurologist, has, has, etc....)
3. Record, mark and save the recording: Save as Narrow Slow Saccade and the subject's name.

Repeat these same instructions, slowly reading and focusing on each word, for the Spaced Word paragraph (words space far apart in the document), the four skeleton document (each line having the word "skeleton" spaced throughout a line four times, and the five skeleton document (each line having the word "skeleton" space throughout five times).



Methods Figure 3. Saccadic eye movement of a subject reading from documents with different numbers of words per line. EOG recorded from a subject reading the four skeleton paragraph versus the five skeleton document. Note the different number of steps in the four versus the five skeleton EOG.

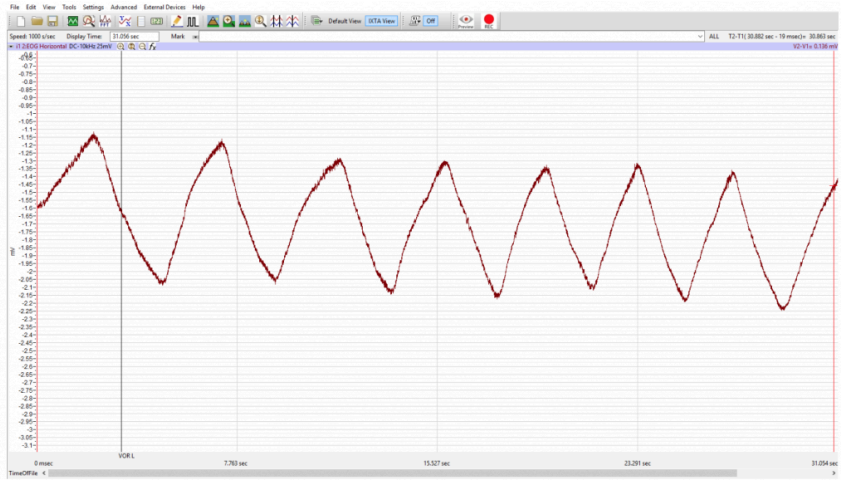
Exercise 2: Vestibular Ocular Reflex (VOR)

Aim: To demonstrate the type of electrical activity that occurs in the muscles as the

subject remains focused on an image or word on a page while rotating his or her head from side to side.

Note: Observe the subject's eye movements as well as the pattern of the recording.

1. Using the Card with VOR printed on it, have the subject focus on the letters VOR as the subject rotates his or her head from side to side.
2. **Inform the subject to avoid any voluntary movements of his or her BODY during the recording**. **Only the subject's HEAD should be moving while he or she is focusing on the VOR card.**
3. Type **"VOR"** in the **Mark box** to the right of the Mark button on the LabScribe3 Main window.
4. **As the subject is focusing on the VOR image and begins rotating his or her head first to the left side then to the right side**. Continue moving head left to right **while keeping their eyes focused on the VOR image. Click Record**. **Click on the Mark** button to **mark the recording**. Have the subject do this for **15 seconds.**
5. After the first cyclic EOG pattern in completed: click on the AutoScale button and observe the subject's eyes as the subject is rotating his or her head.
6. When the subject has finished the exercise **Click Stop**.
7. Select **Save** in the File menu.



Methods Figure 4. the vestibular ocular reflex. Electrooculogram (EOG) while the subject focuses on the VOR image moving their head from left to right while keeping the eyes focused and not moving.

Exercise 3: Pursuit Eye Movement

Aim: To demonstrate the type of electrical activity that occurs in the muscles as the subject follows a moving target vs a stationary target.

Use computers to view the computer-generated image of a moving ball:

For **Horizontal Smooth Pursuit** Test find the file named "Horizontal Ball" located on the P: drive.

1. The subject must position their face **12 INCHES** in front of the screen, **prepare subject to focus** on the LEFT SIDE of the computer screen to begin this exercise. Remind the subject that only their eyes will move in this exercise. You will record activity for **Four Cycles**, a **Cycle** is watching the dot from the **LEFT to RIGHT and BACK TO LEFT**. **Do this Four Times.**
2. Type "**Horizontal Smooth Pursuit**" in the **Mark box** to the right of the Mark button. As the computer program is started, watch the screen and say "RECORDING" when the dots returns to the VERY LEFT HAND SIDE OF THE SCREEN and the subject begins following the target with his or her eyes.

3. CLICK RECORD and Click **MARK** to place the Mark on the recording screen.
4. REMIND the Subject that ONLY the SUBJECT'S EYES should be moving while he or she is following the target.
5. Have subject follow the dot "**LEFT to RIGHT and BACK TO LEFT**" for **FOUR CYCLES**

After the first cyclic EOG pattern in completed,

- **Click** on the AutoScale button
- Observe the subject's eyes as the subject is following the target.

6. When the target has completed FOUR CYCLES. **Click STOP** to halt recording Select **Save** in Your Table's File on the Desktop. Title this "Horizontal Smooth Pursuit and the subject's name.

Vertical Smooth Pursuit

For **Vertical Smooth Pursuit** Test find the file named "Vertical Ball" located on the P: drive.

Have COMPUTER **UNPLUGED** and SET AT EYE LEVEL and **12 INCHES** in front of subject **prepare subject to focus** on the **CENTER** of the computer screen to begin this exercise. Remind the subject that only their eyes will move in this exercise. You will record activity for **Four Cycles**, a **Cycle** is watching the dot from the **TOP TO BOTTOM BACK TO TOP. Do this Four Times.**

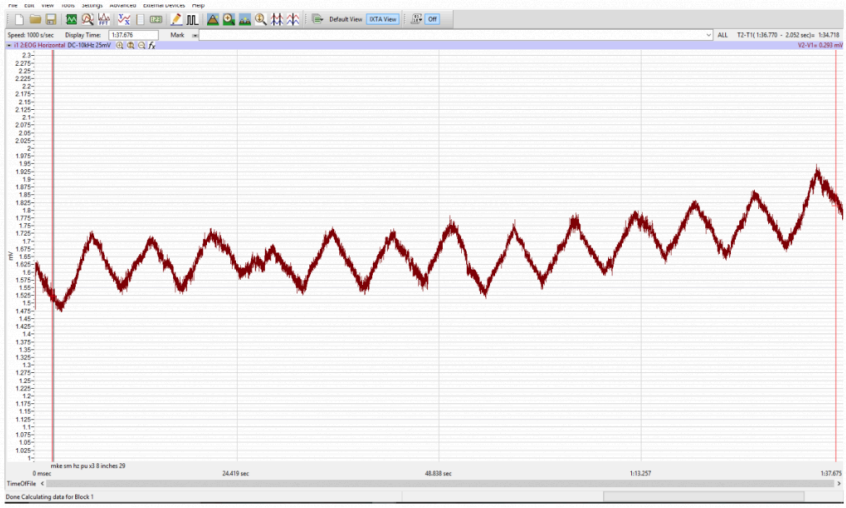
1. Type "**Vertical Smooth Pursuit**" in the **Mark box** to the right of the Mark button.
2. As the computer program is started, watch the screen and say "RECORDING" when the dots returns to the VERY TOP OF THE SCREEN and the subject begins following the target with his or her eyes.
3. CLICK RECORD and Click **MARK** to place the Mark on the recording screen.
4. REMIND the Subject that ONLY the SUBJECT'S EYES should be moving while he or she is following the target.

5. Have subject follow the dot "**TOP TO BOTTOM BACK TO TOP** " for **FOUR CYCLES**

After the first cyclic EOG pattern is completed,

- **Click** on the AutoScale button
- Observe the subject's eyes as the subject is following the target.

6. When the target has completed FOUR CYCLES. **Click STOP** to halt recording Select **Save** in Your Table's File on the Desktop. Title this "Vertical Smooth Pursuit and the subject's name.



Methods Figure 5. Electrooculogram (EOG) of a subject watching the Horizontal Smooth Pursuit video.