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A. Course Overview

PSYC 4052/5052 is a 4 credit course in physiological psychology. The content of the course varies as per the interests of the instructor, but usually includes coverage of neuroanatomy, electrophysiology of excitable cells, and discussion of various sensory systems such as vision, audition, motor, and sensory. The course has as a prerequisite a lower level biological psychology course such as PSYC 2012.

General Course Requirements

The lab comprises one fourth of the course credit. There is no separate grade for the lab. How the lab contributes to the course grade is at the discretion of the course instructor, but typically the lab is worth one fourth of the total points for the course. Consult your course syllabus. Laboratory attendance is mandatory and a missed lab will not be made up. Also, as the lab period is short (2 hours compared to 3 or even 4 in other courses) it is important that you show up on time and that you have read the material for the current lab exercise.

This Manual

Because the interests of the faculty involved with PSYC 4052 vary dramatically, creating a standard manual for the lab is difficult at best. Instead, this manual represents a collection of topics that have been used in the lab over the years. Your course instructor will choose labs that best fit the topics to be covered in lecture. This may also include additional material not included in this manual. Consult your lab syllabus.

The labs in this manual fall roughly into three categories: neuroanatomy, neurophysiology, and neuroscience methods. Neuroanatomy is studied using the sheep brain and is covered in four separate lab periods. These labs were chosen to give hands-on experience to reinforce and clarify anatomy topics presented in lecture. The electrophysiology labs explore the behavior of excitable cells from a hardware, software, and “wetware” perspective. The methods labs give you an opportunity to explore techniques used in neuroscience research.

Persons contributing labs to this manual were asked to include a number of study questions following the lab. Many of these questions provide a means of reviewing the lab material and are discussed within each lab. Some are thought questions, without a right or wrong answer. Some of these questions may require additional research in your textbooks or library.

Lastly, the manual is still evolving. Changes are usually guided by the response of students to the current material. If you have suggestions for improving a particular lab, or have ideas for additional labs, please bring them to the attention of your teaching assistant.
Guidelines for Assignments

Materials turned in for the lab must be neat and legible. You are encouraged to use a word processor for the assignments - every student at this university has access to one. Questions or problem statements should be restated in the write-up. Include a discussion if one is appropriate. Graphs must be done using graph paper and a straight-edge. Label the axes and include a title. Alternately, you may use one of the many plotting programs available (most word processors also have an option for making graphs). You will find that the effort invested in learning to use one of these tools is time well spent.

In general, make the materials you turn in as clear, concise and polished as you can. Unacceptable materials will be returned ungraded. Keep returned materials in a safe place until the end of the semester in case they are needed for score verification.

Animal Use Policy

This course uses vertebrate animals. This includes, but is not limited to, whole animals, animal parts, and animal tissues. In some experiments, death of an animal may result. The use of animals has been minimized where possible through alternate teaching tools such as computer simulations and videotapes, however many aspects of neuroscience cannot be effectively conveyed without the use of animal models. Students are expected to participate in every laboratory.

Laboratory Safety

Safety in the laboratory is mostly an exercise in applied common sense. The main rules in this or any lab dealing with chemicals and/or biological materials are the following:

1) Most of the chemicals used are hazardous and/or carcinogenic. These include the fixatives used for the sheep brains and the histological stains. Gloves will be provided. Be aware that the table surfaces pick up chemicals. Don’t bring food to the lab. Don’t eat or let your pets lick your hands after lab without first washing your hands. If you are pregnant, or have any respiratory problems you may want to wear a surgical mask.

2) For the same reasons, do not bring your pets and/or children to lab.

3) Biological materials and gloves must be properly disposed of in biohazard bags. See your teaching assistant if you have questions.

4) Scalpels are amazingly sharp and will cut fingers if given a chance. This is especially dangerous if they have just been used on fixed tissue. Scissors and dissecting needles are also sharp. Be aware that you may be (more) clumsy when wearing gloves. Know the location of the first-aid kit.

5) Running, horseplay, etc. is always a bad idea.
Academic Ethics

Students are expected to be familiar with the university guidelines regarding academic ethics. A copy of the policies of the College of Arts and Sciences follows:

University of Colorado, Boulder
College of Arts and Sciences
Statement of Academic Dishonesty

The maintenance of the highest standards of intellectual honesty is the concern of every student and faculty member in the College of Arts and Sciences. The faculty is committed to imposing appropriate sanctions for breaches of academic honesty.

Academic dishonesty can occur in many ways. Some common forms of academic dishonesty are:

I. Plagiarism: Each student is expected to present his or her own work. All papers, examinations, and other assignments must be original or explicit acknowledgement must be given for the use of other persons’ ideas or language. Examples of plagiarism as it might occur in term papers, research papers, laboratory reports, and other written assignments are listed below.
   A. Failure to use quotation marks: All work which is quoted directly from a source should be enclosed in quotation marks and followed by taken. Failure to use the quotation marks, even if a footnote source is provided, is plagiarism.
   B. Failure to document ideas: When a student uses one or more ideas from and/or paraphrases a source, he or she must give the exact page or pages from which the ideas or paraphrasing were taken. Failure to provide the exact reference is plagiarism.
   C. False documentation: Falsifying or inventing sources or page references is plagiarism.

Ideas which are part of the general fund of human knowledge (e.g., George Washington was the first president of the United States, Albert Einstein developed the theory of relativity, etc.) need not be documented in student papers. Students are encouraged to ascertain from the instructor in each class what the instructor’s discipline considers to be information included in this provision.

II. Cheating on Examinations: Students are expected to present their own work in all examinations. Some examples of cheating as it might occur in examinations are as follows:
   A. Copying the work of another student during an examination.
   B. Permitting another student to copy one’s work during an examination.
   C. Possessing unauthorized notes, crib sheets, additional sources of information, or other material during an examination.
   D. Writing the answer to an exam question outside of class and submitting that answer as part of an in-class examination.
   E. Taking an examination for another student.
   F. Having an examination taken by another student.
   G. Altering or falsifying examination results after they have been evaluated by the instructor and returned to the student.
III. Other Form of Academic Dishonesty:

A. Possessing term papers, examinations, lab reports or other assignments which have not been formally released by the instructor is dishonest (formally releasing such material means that the instructor has distributed material to the students and has not required that it be returned);

B. Possessing another student’s work without permission.

C. Writing a paper, lab report or other assignment for another student or submitting material written by someone else.

D. Submitting the same paper for two different classes without the explicit authorization and approval of the faculty members teaching those classes (it is immaterial whether or not the classes are taught the same semester).

E. Selling or purchasing examinations, papers, or other assignments.

F. Falsifying University documents for the purpose of altering a transcript or other official University record.

G. Presenting forged or false statements for the purpose of enabling a student to take advantage of such University policies as pass / fail, incomplete, and late withdrawal.

H. Altering another student’s examination, term paper, laboratory work or other assignment.

I. Falsifying data.

IV: Sanctions to be Imposed: Faculty members MUST impose sanctions for violations of academic ethics. In addition, faculty members are REQUIRED to report in writing all breaches of academic honesty to the Associate Dean.

The minimum sanction available to the faculty member is to award an F on the assignment in which the dishonesty occurred. Faculty members may at their discretion impose intermediate sanctions such as awarding an F on the laboratory portion of the course.

In addition to the above range of sanctions, each faculty member has the prerogative of referring a case to the Committee on Academic Ethics with a specific request that the Committee consider imposing additional sanctions such as suspension and expulsion.

In reporting violations of academic ethics to the Associate Dean of the College, faculty members shall state in writing the circumstances surrounding the incident in question, the nature of the evidence collected against the student, the results of the interview with the student in which an allegation was presented to him or her, and the sanction to be imposed. Supporting documentation shall also be provided to the Dean’s office where it will be kept in the permanent record of the student according to College record-keeping policies. The faculty member shall send a copy of the letter to the Associate Dean to students accused so that they are aware of the existence of this record.

Adopted Spring 1983
On Writing a Scientific Paper

In reporting your scientific results, you will be expected to use accepted scientific writing style. The specific format of a scientific paper varies by the journal in which it is published. However, all scientific writing emphasizes conciseness, clarity, and presentation of information in a logical sequence. Although these criteria restrict writing flexibility, bear in mind that the purpose of a scientific report is to convey a specific set of information in an ordered manner. Although it can be frustrating initially, train yourself to write clearly and concisely.

Keep in mind that a simple, direct, and concise writing style is effective. Statements which add little information to a paper should be eliminated. For example, "My mouse did not like the saline injection." or "I was confused on this point," or "It could be argued that...." or "The results are very interesting" could all be deleted from the text without affecting the information presented. Furthermore, statements reflecting value judgments ("The mouse did not like...") should never be included since the "likes" or "dislikes" of the animal have not been experimentally established. After finishing the text of your paper, read it critically and carefully for extraneous ideas, cumbersome phrases, and value judgments. Eliminate all that you find from the text. You should also have a friend critically evaluate the paper, since a person who is not familiar with what you have written can provide helpful information on what is not clear.

All reports are to be typed (12-point font or equivalent) with reasonable margins. All figures should be computer generated (not hand-drawn) and integrated into the report in a consistent fashion. ALL REPORTS SHOULD BE SAVED ON A COMPUTER DISK-- in the event that the hard copy of the assignment is misplaced, the paper can be easily re-printed.

Organization

Most papers are organized as six major divisions, each of which is set off from the others and has a specific function as described below. You should use the following format.

Abstract. Most journals require an abstract at the beginning of the manuscript which lists the purpose and principle results of the study.

Introduction. This section briefly presents background information which orients the reader to the general problem to be addressed by the investigator. All statements of fact are followed by a reference (e.g. "Coulson (1994) found that..." or "Mice become hypoglycemic following cold exposure (Coulson, 1994)". This permits the reader to examine previous reports in the area, if desired. All references cited are listed in the last section of the paper. At the end of the Introduction, give the specific objectives of your investigation.

Material and Methods. Since you have already informed the reader why you are interested in this problem and what specifically you want to do, the material and methods outlines how you are going to do it. This section should be written so that the reader could repeat the experiment, if necessary. Thus, the instrumentation used, concentration of solutions, time intervals, etc. should be included. Most of the important information can be found in your lab manual. There is a tendency to make this section very long by including every minute detail in the experiment. For example, "We placed the animal on the counter top to inject the anesthetic" or "The drug was administered for 5.56 seconds after the
mouse was immobilized". Be aware of this tendency and keep the materials and methods to the bare minimum necessary to repeat the experiment. Do not simply repeat the lab manual - it is acceptable to refer to the manual where appropriate. Always use the metric system (liters, grams, meters, seconds) in scientific writing. Use past tense.

**Results.** In this section you summarize your major results in writing and elaborate on specific points, such as exceptions to the rule or some observation of particular interest. For example, "Cold-exposed mice exhibited at 10% drop in body temperature relative to warm-exposed animals (see Table 2)" or "Yellow male flies sired 10% more offspring than- (see Table 3). These would be appropriate topic sentences in the first paragraph of the results. The topic sentence might be followed by an observation of lesser importance, such as "However, one mouse did not respond to cold exposure". All tables and figures should have a caption explaining that table or figure. Special notations (e.g., cold-exposed mice.-) should be explained. Parametric statistics (nest weight or metabolic rate) are expressed as a mean plus or minus SEM. If you have subjected the data to a t-test, the results can be expressed as a t-value with the degrees of freedom (e.g., t-3.5; d.f.=15) or as a probability (e.g., p<0.05). Such statistics are usually enclosed in parentheses following the appropriate statement of fact. For example, the metabolic rate of cold-exposed mice was substantially higher than warm-exposed (see Table 1) (t-2.4; d.f.=15). Keep in mind that it is the biology which is important in reporting the results--statistical analyses only assist you in making a decision about the data; do not let this section become an extensive discourse on statistics. Finally, refrain from data interpretation in this section.

**Discussion.** This is the section for objective interpretation of your data. Explain the relevance of your results with proper references to the appropriate figures tables. Where possible, compare your findings with other scientific reports. If appropriate, mention the limitations in your techniques or data, but do not turn this section into a sad tale of everything that went wrong. Critically evaluate your results in this section. Since you will be reading general references in this course, they will suffice. You are not expected to do a literature survey.

**Literature Cited (or References).** List in alphabetical order all the references mentioned in the text. Do not include references which you read but which were not mentioned in the paper. Footnotes are not used in scientific writing. Although the specific format for references differs from journal to journal, we will use the following:

Sheep Brain Neuroanatomy

I. External Surface

Materials

- Dissecting Tray
- Ziplock Bag
- Forceps
- Sheep Brain
- Scissors

General Purpose of Neuroanatomy Labs

The next few labs are intended to help you develop an understanding of neuroanatomy. Neuroanatomy includes developing a three-dimensional representation of the brain, understanding the relationships between internal brain structures, and understanding the function of these brain structures. A comprehensive list of brain structures you will be expected to know will be provided. You should work in a way that will help you both identify the brain structures on a real sheep brain and be able to explain their functions. To aid you in learning neuroanatomy you will be supplied with a sheep brain that you may dissect, you can use the interactive brain atlas on the computers, you should utilize your textbooks, and you may repeatedly ask your TA “What’s the function of this brain structure?”

Warning!!! Note the smell of formaldehyde as you pick up your sheep brain. This means formaldehyde is in the air and landing on everything in the room. Would you drink the formaldehyde in the sheep brain buckets? Hopefully not, because not only would that just be gross, it would also make you very sick. For this reason it is imperative that you do not drink or eat in the lab.
Getting Started

Once you obtain a sheep brain on a dissection tray and have a pair of forceps and scissors you are ready to start the dissection. The sheep brain has three layers of protective coverings on them. These protective layers are called meninges. At the rostral end of the sheep brain poke a hole in the meninges along the midline. Try to only go through the meninges and not damage any brain tissue.

![Diagram of sheep brain with meninges and cerebellum marked]

Continue posterior along the midline cutting the meninges. As you near the posterior end of the brain notice a “bony” area lying on the midline in the shape of an elongated triangle. Cut around this area on both sides. Now remove the meninges from the cerebellum. We next want to remove the “bony” area that lies between the cerebral hemispheres and the cerebellum. To do this, orient your scissors so that they are perpendicular to the plate. Slightly pull back the cerebellum so that you can slip your scissors in and cut the bone on each side. If you cut through the entire bone, it will easily peel off.

![Diagram of sheep brain with bone plate and cranial nerves marked]

Flip your brain over to the ventral side. On this side you will have to be extremely careful not to pull off the cranial nerves. Starting at the posterior end (spinal cord) gently lift the meninges 1mm off the ventral side of the brain. Look under the meninges for cranial nerves that are being pulled up with the meninges (they look like tiny pieces of linguini). You want to cut the cranial nerves as close to the meninges as you can, i.e. leaving as much of the nerve attached to the brain as possible (remember they are in pairs, one on each side). Continuing anterior you will run into the largest
cranial nerve called the trigeminal nerve (its about the diameter of a pencil) just cut the meninges around it leaving the nerve in place. Right smack in the middle of the brain on the ventral surface you will see a gray ball (just posterior to the optic nerves), this is the pituitary. The pituitary is an endocrine gland which releases hormones into your blood stream. The pituitary is NOT part of the brain, but is attached to the brain by a tiny stalk called the infundibulum. Look at the location of the pituitary now because soon it will be gone. As with many of the cranial nerves, the pituitary will be lost to the causalities of dissection. At this point continue the dissection of the meninges until you reach the very anterior part of the brain. The olfactory bulbs are attached to the meninges and should be kept as whole as possible while removing the meninges.

The following structures are covered in this lab. You are responsible for their location and function.
External Structures

Meninges

1) Dura
2) Arachnoid Substance
3) Pia Mater

Telencephalon

Cerebral Lobes

• Frontal
• Parietal
• Temporal
• Occipital

Sulci/Fissure

Longitudinal (cerebral) fissure
Central sulcus
Lateral (sylvian) sulcus
Rhinal sulcus

Gyri

Precentral gyrus
Postcentral gyrus
Superior temporal gyrus
Parahippocampal gyrus (Pyriform)

Olfactory bulbs
Diencephalon

Mamillary bodies
Optic tracts
Optic chiasm
Infundibulum

Mesencephalon

Cerebral peduncles

Metencephalon

Pons
Cerebellum
  • Vermis
  • Cerebellar hemispheres
    • Intermediate
    • Lateral

Mylencephalon

Pyramids
Medulla Oblongata

Special Structure

Pituitary
FIGURE 8-3
Sheep brain (ventral view).
Sheep Brain Neuroanatomy

II. Cranial Nerves and Midline Structures

Purpose

In this lab you should review the structures from the first dissection, locate and identify the cranial nerves, make a mid-sagittal cut and identify the important midline structures.

Cranial Nerves

While little time is spend studying the twelve pairs of cranial nerves, their function can not go unmentioned. The cranial nerves and the spinal cord are the only routes by which the brain receives and sends out information. Physical stimuli (i.e. photons of light, waves of air pressure, vaporous chemicals, etc...) are constantly bombarding our bodies. Our external receptors are able to detect these physical stimuli and convert them into electrical impulses which travel to the brain via the cranial nerves and spinal cord. Once these signals reach the brain they can alter the chemical and electrical activity in certain brain areas to result in a sensory perception (i.e. vision, sound, smell, ect…). Imagine cutting your optic nerve, your eye still has receptors that respond to light and your brain still has neurons that make up your visual cortex, but there is no communication between them, therefore you’re blind. Your whole perception of the world comes from external stimuli that are detected by receptors, converted into electrical signals (action potentials), travel to your brain via the cranial nerves and spinal cord, and result in chemical changes in specific brain regions. Damage anywhere along this pathway could wipeout or impair a sensory modality (vision, hearing, smell, ect…). Since the importance of the cranial nervous should now be obvious, you should know them each by name and the type of information they carry.

Clarification of some vocabulary may be helpful at this time. You should already know a neuron is made up of dendrites, a cell body, an axon, and a bouton. A group of cell bodies in the brain is called a nucleus (“gray matter”). A group of cell bodies in the periphery is called a ganglion (i.e. dorsal root ganglion). A bundle of axon fibers in the brain is called a fasiculus, tract, or peduncle (“white matter” due to myelin). A bundle of axon fibers in the periphery is called a nerve. Finally, afferent describes information send to the brain, while efferent describes signals coming from the brain.

Cranial nerves are therefore part of the peripheral nervous system. Some cranial nerves carry afferent information (sensory), others carry efferent information (motor), and a few carry both afferent and efferent information (different neurons carry afferent and efferent information, but their axons run in the same nerve). This information is important when determining what type of deficits a person will have if a cranial nerve is cut or damaged by a stroke.
Getting Started

Since a number of the cranial nerves are so delicate they will be lost during the removal of the meninges, we divided the cranial nerves into two groups, one that you will be responsible for identifying and knowing their function and a second group which you will only be tested on their function.

Once you have identified the cranial nerves divide into groups of three or four and pick one sheep brain to make a mid-sagittal cut on (the other brains will be dissected over the next two weeks). Take “the chosen brain” and one of the large blades, ventral side up make a mid-sagittal cut (start at the rostral end and draw the knife towards you in one motion – do not use a sawing motion !). Now using your two sagittal surfaces identify the midline structures.
Cranial Nerves (＃)

(Anatomy & Function) (Only Function)

Optic nerve (2) Olfactory nerve (1)
Oculomotor nerve (3) Vestibulococlear nerve (8)
Trochlear nerve (4) Accessory nerve (11)
Abducens nerve (6) Hypoglossal nerve (12)
Trigeminal nerve (5) Glossopharyngeal nerve (9)
Facial nerve (7)
Vagus nerve (10)

Mid-Sagittal Slice

Ventricular System:

- Lateral Ventricle
- Third Ventricle
- Cerebral Aqueduct
- Fourth Ventricle

Septum Pellucidum

Choroid Plexus

Commissures

- Anterior Commissure
- Posterior Commissure
- Corpus Callosum

Telencephalon

Fornix

Cingulate Gyrus
Diencephalon

Thalamus
Hypothalamus

Mesencephalon

Tectum

• Superior Colliculus
• Inferior Colliculus

Pineal body

Metencephalon

Arbor Vitae

_Old Structure to Identify_ 

Telencephalon

Frontal lobe
Parietal lobe
Occipital lobe
Central fissure
Precentral gyrus
Postcentral gyrus

Diencephalon

Mammillary body
Optic chiasm

Metencephalon

Pons
Vermis of Cerebellum
Mylencephalon

Pyramids
Medulla oblongata
Sheep brain; cranial nerves.
Sheep Brain Neuroanatomy
IIIa. Coronal Structures

**Purpose**

In this lab, you will study brain anatomy from coronal sections. Many of these structures, you will have already seen before from another point of view; for these brain regions you will be able to see their medial – lateral and dorsal - ventral location and what brain regions surround them. There will also be many new brain regions that were not seen in the previous labs.

**Fiber Tracts**

As you observe these sections, notice the contrast between gray matter (cell bodies) and white matter (myelinated axons). One landmark which contains many important fiber tracts is the **internal capsule**. It is the white matter located lateral to the thalamus and medial to the lentiform nucleus. The internal capsule is a massive layer of white matter containing projection fibers connecting the cerebral cortex with various subcortical structures. Many of the fibers are communicating information between the thalamus and the cortex, others start in various motor cortex regions and project through the internal capsule down to the ventral part of the brain where they make up the cerebral peduncles. When these fibers hit the level of the cerebellum they send collateral projects around to it, these collaterals form the pons. Some fibers synapse at nuclei in the brainstem, while others form the pyramidal tract that projects down the spinal cord synapsing on alpha-motor neurons which innervate distal muscle (i.e. fingers & wrist), controlling fine motor movement (writing, eating, picking up objects, ect…).

**Getting Started**

In this lab, you will make coronal sections through one of the sheep brains you saved from the last lab period. The first coronal slice should be made through the rostral portion of the optic chiasm. The second coronal slice is made through the cerebral peduncles at the level of the oculomotor nerves. In these two slices you should be able to identify the coronal structures, you may need to share brain slices between tables in order to identify all the structures. Again, use a large blade and NO SAWING THE BRAIN!
Coronal Slices

Level of Optic Chiasm

Caudate nucleus
Putamen
Globus pallidus
Internal capsule
Amygdala

Old Structures

Longitudinal fissure
Corpus callosum
Cingulate gyrus
Lateral ventricle
Septum pellucidum
Third ventricle
Thalamus
Hypothalamus
Optic chiasm
Fornix

Level of caudal Cerebral Peduncles

Hippocampus
Substantia nigra

More Old Structures

Pineal gland
Superior colliculus
Posterior commissure
Cerebral peduncles
Parahippocampal gyrus
Sheep Brain Neuroanatomy

IIIb. Horizontal Structures

Purpose

The purpose of this lab is not to learn more brain structures, but to view the brain regions you have seen in past labs from a new perspective. Horizontal sections are used to see the rostral – caudal layout of the brain. This is the lab where you should review the external structures, mid-sagittal structures, and coronal structures and try to develop a three dimensional map of the brain. This lab is meant to be a review for the lab practical, so spend your time wisely.

Getting Started

Using a large blade make a horizontal slice at the level of the superior colliculus.
Neurophysiology
IV. Electrophysiology of the Neuron
Software Simulation

Materials

NeuralSim Software - A Computerized Interactive Study Tool [1]

Purpose

To investigate the quantitative behavior of action potentials and post-synaptic potentials and their qualitative changes in response to different membrane properties and ion concentrations.

Introduction

The dynamic nature of excitable cells makes it difficult to fully appreciate their behavior using standard textbook approaches. In this lab we will investigate action potentials and synaptic potentials using a computer simulation. The simulator accurately reproduces the behavior of excitable cells based on the mathematical model of Hodgkin and Huxley [2] and presents the results graphically. This will allow you to concentrate on the concepts without getting tied up in the mathematical details.

We will be using the APSIM software, which allows you to simulate the stimulation of an excitable cell. We will introduce its basic function and describe the menu options you will need for today’s lab, after which you should work through the guided exercises provided. These exercises should be completed and turned in at the end of the lab. When you are finished with the required exercises, you may go back and explore other aspects of the simulator if you wish to do so (a list of possible topics is listed at the end of the handout). A second program is included with NeuralSim, PSPSIM, which you are encouraged to try if you have time. This program simulates the behavior of a post-synaptic cell in response to excitatory and inhibitory synaptic potentials. The operation of the two programs is very similar, and you should have little trouble using PSPSIM once you understand APSIM.

APSIM - Action Potential Simulator

The APSIM software allows you to simulate the stimulation of an excitable cell and investigate quantitatively the behavior of the action potential. Behind the scenes are the actual equations derived by Hodgkin-Huxley in their now-classic experiments on the giant squid axon.

The APSIM main window displays the results of running a given simulation. This consists of a stimulating current pulse at bottom (in blue) and plot of the membrane potential. The

1 Contributed by M. Jones, Spring 1996
stimulus strength and duration can be adjusted using the slider bars. To the left of the main display are the three buttons that control simulation execution: Erase, Run, and Halt. Run begins a simulation run. Halt stops a running simulation. Erase clears the screen.

APSIM also has a number of pull down menus for changing membrane properties, making measurements, plotting additional parameters and so forth. Many of these features will not be used in today's lab; those important for the lab exercises are shown below in **boldface**:

**File**
- Close
- Run
- Halt
- **Quit** - Quit the Program

**Edit**
- **Mode**
  - **Membrane** - Set active or passive membrane
  - **No. Pulses** - Specify one or two stimulating pulses

**Params**
- Maximal Conductances
- Ion Concentrations
- Gating Kinetics
- **Timebase** - Expand or contract the time scale of the main window
- Plot Scaling
- **Reset All Params** - Set all parameters to their startup values

**Plots**
- Membrane Currents
- Membrane Conductances
- Channel Gates
- **Measure** - Bring up the measurement window
- Experiments
APSIM Exercises*
Name: _____________________________
Student ID: ________________________

Notes:

1) In these exercises, current values are measured in µAmps, and are listed at the right of the slide bar, whereas potential is measured in mV, and is measured using the cross-hairs brought up by the Measure menu. Any numerical answers you provide must include the correct units!

2) Note that the membrane resting potential is given at the left of the display (under the control buttons) labeled “R.P.”. The 55 mV and -80 mV listed at the left of the main window in bold represent the Nernst potentials of sodium and potassium, respectively.

3) You may need a calculator for some of the questions. If you don’t have one with you, select the “calculator” application from the apple menu. Ask your TA if you need help.

A. Threshold Potential

Determine the neuron’s threshold potential using a 0.5 msec stimulating current pulse. (Make sure you have selected Active Membrane from the Mode menu. Choose the 1 pulse option from the Mode menu and make sure the timebase is set to 2 msec (use the Time Base item in the Params menu if necessary). Use the Stimulus Strength scroll bar to adjust the strength (i.e. amplitude) of the current pulse until the pulse just elicits an action potential.

A.1) What is the amplitude of the smallest current pulse that elicits an action potential? ______________________

To determine the membrane threshold potential, decrease the strength of the stimulus by one “click” so that stimulation fails to elicit an action potential, open the Measure window from the menu bar and use the cross hair to read the voltage at the end of the 0.5 msec stimulus.

A.2) What is the value of the membrane threshold potential? ____________________

Increase once again the current pulse so that an action potential is generated. Open the Membrane Conductances and Membrane Currents windows from the Plots menu. This will display the changes in sodium and potassium conductances and currents over the course of the simulation, and show you how changes in conductance control the rising and falling phases of the action potential. The Membrane Conductances displays sodium permeability in red, and potassium permeability in blue. Click “View Vm” to have APSim include membrane potential in this plot. The Membrane Currents window shows sodium current in red and potassium current in blue. Down is current flow into the cell (i.e. depolarization). The green line is capacitive current and may be ignored, as can the small spike that occurs in the sodium current. Drag the windows around until they line up underneath one another. You may want to make sketches of these windows, which you can refer to outside of lab.
A.3) Why does sodium conductance rapidly decline after the action potential reaches its peak?

A.4) Why does sodium current continue to increase even as the sodium conductance declines?

A.5) Why does the K current decline more rapidly than the K conductance?
B. Parameters of the Action Potential

Measure the peak voltage of the action potential by centering the cross hairs on the peak.

B.1) What is the value of the action potential peak? ______________________

B.2) Why doesn’t this value equal $E_{Na}$?

Now measure the most negative potential reached during the hyperpolarization-overshoot that follows repolarization of the action potential.

B.3) What is the value of the membrane potential? ______________________

B.4) Why doesn’t this value equal $E_{K}$?

B.5) Why is this value more negative than the resting potential?

The duration of the action potential is defined as the time it takes for the action potential to go from 50% of its full amplitude on the rising phase back to this potential during the falling phase.

B.6) Compute the amplitude of the action potential. This is equal to the peak value (question B.1) minus the resting membrane potential (R.P.) ______________________

B.7) What value of the membrane potential represents 50% of the action potential amplitude?

B.8) Using the cross hairs, determine the latency (t) at which the membrane potential reaches the value obtained in B.7 during the rising phase: ______________________
B.9) Using the cross hairs, determine the latency (t) at which the membrane potential reaches the value obtained in B.7 during the falling phase: ____________________

B.10) What is the duration of the action potential? ____________________
C. Refractory Period

Set the 2 pulses option from the Mode -> No. Pulses menu. Set the Time Base to 4 msec using the Params -> Time Base... option under the Params menu.

Adjust Pulse #1 so that it is 30 µAmps in amplitude and 0.5 msec in duration. Then, click on the Pulse # button in the main window (at the lower left side of screen above the current pulse scroll bars) until the label on the current pulse scroll bar reads Pulse 2. Set the Pulse 2 amplitude to 100 µAmps and its duration to 1.0 msec. Click on the Pulse # button until the current scroll bar label reads Interpulse Interval. Start with an interpulse interval of 5 msec. Click Run.

C.1) How many action potentials are generated? _________

Keep increasing the interpulse interval in steps of 1 msec until you elicit an action potential with the second pulse, at which point refine your adjustment of the interpulse interval to 0.2 msec increments. For our purposes, define the refractory period to be equal to the interpulse interval.

C.2) What is the value of the refractory period? _________________________________

C.3) What would the maximum firing rate (number of action potentials per second) of this neuron be? __________________________

References


ANSWERS:

Question A.1)

18.20 µAmps

Question A.2)

-55 mV

Question A.3)

The rising phase of the action potential is generated by the opening of voltage sensitive Na\(^+\) channels in the membrane that allow Na\(^+\) to move down its concentration gradient (established by the Na\(^+\)/K\(^+\) pump) and into the cell. However, when the action potential reaches its peak, the voltage sensitive Na\(^+\) channels close and, thus, Na\(^+\) conductance is drastically decreased.

Question A.4)

The answer to this question is based on the premise that current (ionic flow) is a function of conductance (channels) plus driving force (electro-chemical gradient).

The question states that Na\(^+\) conductance is declining while Na\(^+\) current is increasing. What this means is that the voltage sensitive Na\(^+\) channels are closing (decreasing conductance), yet more Na\(^+\) ions are flowing across the membrane (increasing current). In the above premise, the only variable left that could be responsible for increasing Na\(^+\) current is the electro-chemical gradient. Knowing this, we can proceed with the answer to the question.

Na\(^+\) is under the largest chemical pressure to enter the cell at the very beginning of the action potential because the Na\(^+\)/K\(^+\) pump has been busy working to keep the majority of Na\(^+\) on the outside of the cell. The question is asking about the later stages of the action potential when Na\(^+\) conductance is declining, so we can eliminate chemical pressure as a reason for the increasing Na\(^+\) current.

That leaves the electrical gradient as the probable culprit (something must be drawing more Na\(^+\) into the cell). To explain this phenomenon, we must consider another ion species, namely K\(^+\). At the end of the rising phase of the action potential, the voltage sensitive K\(^+\) channels begin to open (the delayed rectifier). This lets K\(^+\) ions flow down their concentration gradient (also established by the Na\(^+\)/K\(^+\) pump) and OUT of the cell. Because K\(^+\) is a positive ion, when it flows out of the cell, the inside of the cell becomes more negative. This increase in negativity draws more positive Na\(^+\) ions into the cell despite decreasing Na\(^+\) conductance. Here you have the answer to this question.

Question A.5)
This question is also based on the premise that current (ionic flow) is a function of conductance (channels) plus driving force (electro-chemical gradient).

Unlike question A.4, this question states that K⁺ conductance is declining very slowly, yet K⁺ current is declining at a much faster rate. Transforming this into physiology, we know that K⁺ channels are closing very slowly yet the number of K⁺ ions flowing across the membrane is decreasing at a much faster rate.

With this information, we again know that the question hinges on K⁺’s electro-chemical gradient. The longer that the voltage sensitive K⁺ channels are open, the more time K⁺ has to diffuse across the membrane and approach its equilibrium potential. By the time the voltage sensitive K⁺ channels begin to close (and we observe a very slowly decreasing K⁺ conductance), the majority of K⁺ ions have already diffused across the membrane so there is far LESS pressure on the remaining K⁺ ions to flow. Less pressure equals less movement, and less movement equals less K⁺ current!

**Question B.1)**

44.5 mV (+/- 1 mV)

**Question B.2)**

If we did assume that the peak voltage of the action potential was equal to E₉, we would not be considering how other ions species affect action potential dynamics, namely K⁺.

The peak voltage of the action potential doesn’t equal E₉ because voltage sensitive K⁺ channels begin to open BEFORE the voltage sensitive Na⁺ channels begin to close. Thus, toward the top of the rising phase of the action potential, positive Na⁺ ions are rushing into the cell at the same time as positive K⁺ ions are rushing out of the cell. Where these two ionic flows balance out is where the peak of the action potential is found.

**Question B.3)**

-78.8 mV (+/- 1 mV)

**Question B.4)**

Again, we MUST consider the dynamics of all of the ion species involved in the action potential. The hyperpolarization-overshoot phase does not equal Eₖ because K⁺ is not the only ion in motion, and its effects are tempered somewhat by the movement of Na⁺ into the cell and Cl⁻ out of the cell.

**Question B.5)**

There are two potential reasons for this overshoot.

**FIRST**, the most negative potential reached during the hyperpolarization-overshoot phase is more negative than the resting potential because the resting potential is generated by
the Na\(^+\)/K\(^+\) pump and leakage currents, whereas the hyperpolarization-overshoot phase is created almost entirely by K\(^+\) moving down its concentration gradient and OUT of the cell through voltage sensitive K\(^+\) channels. The reason that hyperpolarization “overshoots” the resting potential is because the voltage sensitive K\(^+\) channels close too slowly to keep the membrane potential from stopping at the resting potential. This delayed closing allows the membrane potential to approximate \(E_K\) which is more negative than the resting potential.

SECOND, the Na\(^+\)/K\(^+\) pump speeds up during the course of an action potential thereby causing the hyperpolarization-overshoot.

**Question B.6)**

\[44.5 \text{ mV} - (-62.7 \text{ mV}) = 107.2 \text{ mV}\]

**Question B.7)**

\[
\frac{107.2 \text{ mV}}{2} = 53.75 \text{ mV}; \quad -62.7 \text{ mV} + 53.75 \text{ mV} = -8.95 \text{ mV}
\]

**Question B.8)**

4.31 msec\(^*\) to reach -9.1 mV

\(^*\)Note: The resolution of the measurement device precludes an accurate measurement!

**Question B.9)**

5.78 msec to reach -9.1 mV.

**Question B.10)**

Duration of the action potential is defined as the time it takes for the action potential to go from 50% of its full amplitude on the rising phase back to this potential during the falling phase. Thus, to find the answer, you must take your answers from questions B.8 and B.9 and subtract them. The positive number (no such thing as negative time), is the duration of the action potential in msec.

\[5.78 \text{ msec} - 4.31 \text{ msec} = 1.47 \text{ msec}\]

**Question C.1)**

1

**Question C.2)**
The refractory period is the time following an action potential during which another action potential CANNOT be generated. In this simulator, the refractory period is 5.40 msec (with an interpulse interval of 5.60 msec another action potential IS generated, so this is outside the refractory period).

**Question C.3)**

Assuming that another action potential can be generated every 5.60 msec (remember that the refractory period is 5.40 msec), and there is 1000 msec in a second, we know that, at most, 178 action potentials can be generated in one second.

"The actual number is 178.5714286, but we MUST round down to the nearest whole number because there is no such thing as 57% of an action potential in physiology (remember, it is an all-or-none phenomena).
Neurophysiology

V. Neurotransmitters and the Autonomic Nervous System:
A Modified Loewi’s Experiment

Materials

Latex gloves
Bull Frog
Dissecting tools
MacLab\textsuperscript{©} Data Acquisition Equipment and Chart software
Ringer syringe with 26 gauge needle
0.05M epinephrine syringe with 26 gauge needle
0.01M acetylcholine syringe with 26 gauge needle
Acetylcholine and atropine sulfate syringe with 26 gauge needle

Purpose

To investigate the effects of the sympathetic and parasympathetic divisions of the autonomic nervous system on the heart by exogenously administering different neurotransmitters. To pharmacologically demonstrate the effects of an antagonist on a neurotransmitter system.

I. Introduction

Intercellular Chemical Transmission

In 1920 Otto von Loewi provided the first empirical evidence that chemical substances are responsible for the bulk of communication between individual neurons and neurons and their target organs (Appendix A is an excerpt from Loewi’s own notes about his revolutionizing discovery). Scientists have since discovered, named, and identified the effects of many of these endogenous (internally produced) substances. Methods have also been developed that permit efficient purification and/or synthesis of these substances so that scientists can purchase these exact chemicals through distributors (much like Christmas shopping from the Eddie Bauer catalog), and administer them to biological preparations under very precise conditions. We will be taking advantage of the exogenous application of two neurotransmitters, acetylcholine and epinephrine, to study their effects on the vertebrate heart. The reason we will use these two specific transmitters will become apparent momentarily, until then, we need to understand some features of cardiac anatomy and physiology.

\footnote{Contributed by Kurt D. MacDonald, Fall 1997}
The Vertebrate Heart

The vertebrate heart primarily consists of cardiac muscle. This muscle, like ordinary muscle, conducts impulses, and it is this electrical activity that signals the heart to contract. While you might expect that this electrical signal is generated in the central nervous system, it is actually initiated by a specialized cardiac structure called the *sinoatrial (SA) node*. The SA node, located in the wall of the right atrium just below the superior vena cava (Fig. 1), is composed of a bundle of specialized cardiac cells that are inherently rhythmic. That is, even if all neural input to this area is eliminated, it will continue to initiate impulses at regular intervals. Furthermore, because the SA node’s inherent rate of depolarization is greater than that of ordinary cardiac muscle, its activity initiates mechanical contraction of the entire heart. Because of this property, the SA node is sometimes called the heart’s *pacemaker*.

Each time an impulse is generated in the SA node, it spreads through the muscle fibers of both atria exactly like an action potential travels down the length of an axon. The presence of *electrotonic synapses* (gap junctions) between all cardiac muscle cells ensures that this conduction is both exceedingly fast and reliable (so your heart never misses a beat).

As the stimulated atria are contracting, the action potential enters another specialized cardiac structure in the right atrium: the *atrioventricular (AV) node* (Fig. 1). As the action potential enters the AV node its conduction slows markedly. This delay permits the atria to fully contract before the impulse initiates ventricular contraction. Thus, the AV node is responsible for establishing the sequence between atrial and ventricular contractions. Once the electrical impulse has passed through
the AV node, it is widely distributed to the ventricles by way of an elaborate conduction network comprised of the AV bundle and Purkinje fibers (Fig. 1).

From the information just presented, it would be logical to assume that normal heart functioning does not require any neural input. It is true that the heart is myogenic (generates its own inherent rhythm), however it also receives rich innervation from the sympathetic and parasympathetic divisions of the autonomic nervous system (Fig.2). It is this input that adjusts the heart’s internal rhythm.

FIGURE 2

Sympathetic control of the heart originates in the cardioaccelerator center of the medulla and reaches the heart by way of sympathetic fibers in the middle, superior, and inferior cardiac nerves. When sympathetic pathways are stimulated they release the neurotransmitter norepinephrine from their long postganglionic cells. When norepinephrine is released from sympathetic terminals that innervate the heart, it primarily activates β-adrenergic receptors. These receptors influence the heart in a variety of ways. When the β-adrenergic receptors of the heart are active they increase the heart muscle’s Ca²⁺ current. This causes an increase in the heart’s force of contraction. Additionally, β-adrenergic
activation causes depolarization of the SA node. This, in turn, causes the heart to beat faster. The effects of norepinephrine on the heart can be seen in figure 3.

Parasympathetic impulses from the *cardioinhibitory* center in the medulla decrease heart rate and cardiac contractility. This inhibitory action is mediated by the release of acetylcholine from the vagus nerve (cranial nerve X). Free acetylcholine acts on muscarinic receptors which hyperpolarize the cells of the SA node and slow the conduction of the action potential through the AV node. This slows heart rate. Acetylcholine also decreases Ca\(^{++}\) influx which lowers the heart’s force of contraction. The effects of vagal nerve stimulation, and hence acetylcholine release, can be seen in figure 4.

![FIGURE 3](image)

![FIGURE 4](image)

At any given time, both divisions of the autonomic nervous system are influencing heart function. However, in a normal resting heart (you sitting in your Lazy-boy reading this lab) the influence of the parasympathetic division is greatest. Thus, when the heart is at rest, we say that the *predominant tone* is parasympathetic. It is only with additional sympathetic activation, the so-called fight-or-flight response (you frantically reading this lab 1 minute before your quiz) that the effects of the sympathetic division are paramount.

In this experiment you will use the MacLab hardware and software to monitor the heart’s response to injections of different chemical substances. This recording will allow you determine heart rate and force of contraction. In the first condition you will administer a Ringer solution (a biologically inert solution) and quantify the heart’s response. In the second condition you will administer an injection of 0.05M epinephrine while monitoring heart function. The third condition involves applying 0.01M acetylcholine to the heart. In the final condition you will apply a mixed solution of acetylcholine and the cholinergic antagonist atropine sulfate.
II. Experimental Setup

Some of the data acquisition hardware you will use in this experiment is identical to the equipment used in the introductory dipole experiment. It consists of the MacLab/400 unit, ETH-250 bridge/bioamplifier, and the Macintosh PowerPC. A new component of hardware is the displacement transducer. As its name implies, this apparatus transforms physical displacement (movement) into an electrical signal that the computer can read.

The data acquisition software required for this lab is also new. In this lab you will be using MacLab’s Chart program. This software emulates an old fashioned chart recorder--an apparatus that records changes in a preparation over time by moving a pen up and down while paper is fed through the machine at a constant speed. (You may be most familiar with the use of chart recorders in the field of geophysics—they are the machines often shown wildly responding to the tremors produced by earthquakes).

III. Method

Because of the myogenic property of the heart, we can perform this experiment on a dead frog. However, the frog must be recently deceased for the experiment to be successful. Your TA will perform a humane method of euthanasia; the rest of the experiment is up to you.

Heart Exposure

We will be performing this study in vivo (within the body), so you will need to CAREFULLY expose the beating heart.

1) With the frog lying on its back (ventral-side up), use a sharp scalpel to make a small opening in the tough outer skin near the rostral boundary of the sternum. Place a tip of the scissors into this opening and make a longitudinal incision that extends along midline to the level of the lower-abdomen. This cut should only pass through the outer skin.
2) Using the scissors, make two perpendicular cuts (one at each end of the initial cut) that will permit you to fold the outer skin away from the midline as two separate flaps (very fine use of the scalpel may be necessary to separate the tough outer skin from the underlying tissue). Examine the beautiful arterial system present on the underside of these flaps.
3) The frog heart, similar to our mammalian heart, is located under the sternum and is protected by a ribcage. To approach the heart, we will begin by making a small incision in the wall of the abdomen at the level of the frog’s imaginary navel (frogs don’t have umbilical cords, hence they have no navels). Extreme care must be taken to avoid puncturing the delicate tissue and organs that underlie the abdominal musculature.
4) Using scissors, extend this cut in the rostral direction until you approach the ribcage (do not damage the scissors by attempting to cut bone). Near the rostral end of this cut, near the base of the rib cage, make two transverse/horizontal cuts in the abdominal wall (if you are confused where to make this cut, ASK your TA). At this point you should have a clear view of the frog’s liver, stomach, intestines, and egg sac (if it is female). You should also be able to see the beating heart if you gently lift the sternum and look under the ribcage.
5) After locating the heart, and ensuring yourself that you will not damage it, use the bone snips to cut up both sides of the rib cage. Separate these cuts with sufficient distance so that you will have ample space around the heart in which to work.
6) Remove the flap created in step #5. The myogenic heart, encased in an opaque membrane (pericardial sac), should be in clear view! (You can use a wooden stick to spread the chest cavity open so that you have a larger working area around the heart).

7) The final step involves carefully cutting away the pericardial sac (do not cut any of the heart’s entering or exiting vasculature).

Setting up the MacLab Hardware and Software

1) Make sure that the displacement transducer is plugged into Channel 1 on the back of the ETH-250 amplifier, and that the output of the ETH-250 is connected to the CH1 input of the MacLab unit.

2) Set the Mode to bridge, Gain to 100, and Filter to W.B. (wideband).

3) Double click on the Chart icon in the MacLab folder. After some initialization, the main chart window is shown. By default, Chart displays 8 channels. To the right of the display window for a given channel are some controls for that channel. To the right of channel 2, click and hold on the Channel 2 control and select “Turn channel off…” from the pop-up menu. Repeat this process for channels 3 & 4 (channels 5-8 are off by default).

4) Click and hold on the double line separating the display window for channel 1 and channel 2. Drag this line to the bottom of the screen so that the display for channel 1 occupies the entire screen.

5) Choose Display settings from the Setup menu and click the Always seconds box in the dialog window that appears.

6) To the right of the Channel 1 window, click and hold on the Channel 1 control, and select Input Amplifier from the pop up menu. Make sure that both the Positive and Negative boxes are selected. Gently tap the displacement transducer and note that a response is generated on the display.

7) Decrease the Range to 50 mV and adjust the Input Offset knob on the ETH-250 to center the signal in the display. (Throughout the experiment, there may be times when the display reads out of range. When this occurs, leave the Range at 50mV and adjust the Input Offset to return the signal to within the visible range). After you have done this, click OK.

8) In the main chart window, click on Start in the lower right corner. The display begins to scroll, recording the movement of the displacement transducer. Note that elapsed time (in seconds) is displayed above the Channel 1 control. You may change the sweep rate (i.e. how fast the “paper” is moving in the window) by clicking and holding the arrow box next to the elapsed time.

Linking the Heart to the Displacement Transducer

To accurately quantify changes in heart rate and force of contraction you will be using the displacement transducer. To properly attach the heart to the displacement transducer, use a threaded suture needle to tie the tip of the ventricle to the end of the displacement transducer (don’t worry about the string tension when you are tying the knot, you can adjust the tension by changing the location of the transducer on the ringstand). Raise the transducer on the ringstand to slightly elevate the heart and take the slake out of the suture thread. A signal should be displayed on the chart display.
## IV: Experimental Conditions and Data Worksheet

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<thead>
<tr>
<th>Condition</th>
<th>Delay (Sec.)</th>
<th>Latency of the last peak on the screen (sec)</th>
<th>Latency of the first peak on the screen (sec)</th>
<th>Number of peaks on the screen</th>
<th>Heart Rate</th>
<th>Change in rate from previous condition</th>
<th>Amplitude of the first peak on the screen</th>
<th>Amplitude of the last peak on the screen</th>
<th>Average amplitude of the first and last peak</th>
<th>Change in amplitude from the previous condition</th>
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Changing the Experimental Condition

Begin by pressing **Start** and recording a few minutes of baseline heart function. To quantify heart rate and force of contraction, press the **Stop** button in the lower left corner of the display and make the measurements necessary to complete the appropriate section in the following data worksheet (actual instructions on the measurement procedure follow the data worksheet page). Once these measurements are complete, make an injection and immediately press start (notice that the elapsed time counter has reset; the display may also report that the data is **Out of Range**. If this is the case, don’t panic and **do not** press **Stop**. You may readjust the **Input Offset** while recordings are being made). Complete this series of starts and stops until the data worksheet is complete.

Calculating Heart Rate and Force of Contraction

The data sheet presented on the preceding page is designed to help you complete the calculations necessary to quantify heart rate and force of contraction.

- To record the latency of a peak, place the cursor on the positive point of the peak and read the value (in seconds) from the window above the **Channel 1** control box.
- one helpful tool that allows you to make far more precise measurements is the blowup window. Ask your TA how to locate this window.
- To calculate heart rate, divide the number of peaks by the time elapsed between the first and last peak and then multiply the result by 60. This will yield the number of heart beats per minute.
- To calculate the change in rate, subtract the old rate from the new rate and divide the result by the old rate. Multiply this number by 100 to get the percent change.
- To measure the amplitude of a single peak, place the cursor on the tip of its most positive peak and record its amplitude from the window above the **Channel 1** control box; then place the cursor on the tip of its most negative peak and record its amplitude. The total height of a peak is calculated by adding the absolute value of its most positive and negative peak. Again, the blowup window will be very useful here.
- To calculate the average amplitude of the first and last peak, add their amplitudes together and divide by 2.
- To calculate the change in amplitude, subtract the old amplitude from the new amplitude and divide the result by the old amplitude. Multiply this number by 100 to get the percent change.

V: Lab Report

Your lab report should follow the introduction-methods-results-discussion format introduced previously.

The introduction should be comprised of general information about the sympathetic and parasympathetic divisions of the autonomic nervous system and how these different divisions innervate the heart (i.e. how these fibers get to the heart). In addition, knowing the neurotransmitters used by the postganglionic cells of the parasympathetic and sympathetic divisions, you should hypothesize what effects these systems will have on the heart when they are increasingly activated. Here it would be appropriate to cite references to support your hypothesis (general neuroscience texts are acceptable).

The methods should be relatively straight forward. You need to describe the experimental setup, including general information about the hardware and software. You also
need to describe each of the experimental conditions in sufficient detail that a reader unfamiliar with the experiment could replicate it on his/her own. This, of course, means including quantities, concentrations, delays, and measurement techniques. Remember, no results should be included in the methods section.

The results section should include 2 tables. The first table should include the data from the 5 columns of the data worksheet that pertain to the heart’s rate (latencies, # of peaks, heart rate, and change in heart rate). The second table should contain all of the data from the remaining columns that quantifies the heart’s force of contraction (amplitudes, average amplitude, change in amplitude). In addition to these two properly labeled tables, include a few brief paragraphs describing the results (be sure to refer to your tables in your text).

The discussion section should compare your results with the results you had expected to obtain. Any discrepancies should be mentioned, and you should theorize why they occurred. Additionally, any unusual results should be explained in this section. (Hint: if you know anything about the mechanism of action of war gasses, now would be a good time to compare that to the results of this experiment.)

References


The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at six o’clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o’clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. I have to describe briefly this experiment since its results became the foundation of the theory of chemical transmission of the nervous impulse.

The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beat diminished just as if its vagus had been stimulated. Similarly, when the accelerator nerve was stimulated and the Ringer from this transferred, the second heart speeded up and its beat increased. These results unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances which, in turn, cause the well-known modifications of the function of the heart characteristic of the stimulation of its nerves.

(Taken from Kandell & Schwartz, 1991)
Neurophysiology
VI. Frog Gastrocnemius: The Dynamics of Motor Control

Materials

- Latex gloves
- Bull Frog
- Dissecting tools
- Suture thread
- Fish hook
- Ring stands (2)
- Femur clamp
- MacLab Data Acquisition Equipment and Chart software
- Force & Displacement transducers
- Ringer solution in 25 cc syringes

Purpose

Using the frog gastrocnemius muscle, this lab is designed to investigate how the brain controls muscle movement. In doing so we will gain a better understanding of the anatomy and physiology of striated muscle and the neurons nervous system that work together in order to produce very complex biomechanical movements.

VIII. Introduction

Voluntary movement is essential for healthy survival, and effective voluntary movement requires adequate muscle mass and an intact nervous system. In a normal adult, skeletal muscle comprises approximately 50% of total body weight, and the importance of neuromuscular modulation is evident in spinal damaged or diseased populations.

The Motor Unit

The motor unit is the functional unit of skeletal muscle, and it can be divided into four fundamental components(Kandel, et al. 1991). These components include the cell body of the motor neuron, its axon, the neuromuscular junction, and the muscle fibers innervated by that neuron (see Fig. 1).

The cell body of the motor neuron lies in the gray matter of the ventral horn of the spinal cord and is, therefore, a component of the central nervous system (CNS). Its myelinated axon exits the spinal cord and travels to the muscle via a peripheral nerve. Along its length, the axon can bifurcate (split into multiple branches), and each collateral (branch) of the axon innervates a different muscle

Contributed by Kurt D. MacDonald, Fall 1997
fiber. The number of muscle fibers innervated by a single motor neuron, and hence the degree of axonal bifurcation, varies greatly depending on the function of a given muscle. For example, in muscles responsible for very precise movements—like the fine muscles of the eye and hand—a single motor unit may consist of only three to six muscle fibers. However, a single motor neuron may innervate 2000 muscle fibers in the large muscles of the trunk and limbs. As a general rule, when fewer muscle fibers are supplied by a single motor neuron, the muscle can produce more precise movements (Thibodeau, 1990).

Like most neuron-to-neuron communication, there exists a small gap, or synapse, between the axon of the motor neuron and the muscle fiber. This specialized junction is called a **neuromuscular junction** or a **motor endplate**.

![Diagram of neuromuscular junction](From Kandel, et al., 1990)
Striated Muscle Anatomy

Skeletal muscle is composed of bundles of muscle fibers that generally extend the entire length of the muscle (see FIG 2A). They are called “fibers” because of their threadlike morphology (1-40 mm long and 10-100 µm). The plasma membrane of a muscle fiber is called the sarcolemma, and it contains sarcoplasm (cytoplasm) (see FIG 2B). Within the sarcoplasm there are a number of unique anatomical structures that give muscle its unique properties. There is a series of tubules that transverse the muscle fiber called T-tubules. The sarcoplasmic reticulum also occupies a position within the sarcoplasm. It too is a tubular system but these tubes run parallel to the muscle fiber and end in small sacs which serve as intracellular sites for Ca\(^{++}\) storage. These two tubular systems intersect at a location called the triad. All of these components are visible in Figure 2B.

Also within the sarcoplasm is a bundle of very fine fibers called myofibrils which extend lengthwise within the muscle fiber sarcoplasm. Myofibrils are composed of proteins which are aligned in a series of repeating units called sarcomeres (see FIG. 2C). A sarcomere is the basic contractile unit of muscle. Stated another way, muscle contraction is achieved by the simultaneous shortening of all sarcomeres within the muscle.

The theory that underlies how each sarcomere shortens is called the sliding filament theory. Detailed information about the molecular mechanism of sarcomere shortening is available in any general physiology textbook, but suffice it to say that shortening is produced by increasing the amount of overlap between thick and thin filaments in the sarcomere (more on this later). Thin filaments, composed primarily of actin molecules, project from the Z-line (see FIG 2D) and overlap the myosin composed thick filaments in the center of the sarcomere. During contraction the filaments do not change length, rather they slide over one another.

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Adopted largely from Thibodeau, 1990.
FIGURE 2  (From Thibodeau, 1990)
Generation of Skeletal Muscle Contraction:

When an action potential reaches the end of a motor neuron’s axon, it stimulates the release of acetylcholine (Ach) from the presynaptic terminal. This chemical transmitter diffuses across the neuromuscular junction and binds to **nicotinic acetylcholine receptors** on the membrane of the muscle fiber. When these Ach receptors are bound, a channel in the sarcolemma opens and allows $\text{Na}^+$ to move into the interior of the fiber and $\text{K}^+$ to flow out of the fiber (see FIG. 3).

![Diagram of skeletal muscle contraction](image)

**FIGURE 3**  
(From Kandel et al., 1990)

The net result of this ionic movement is muscle fiber depolarization. This transient positive deflection in the muscle fiber’s resting membrane potential causes voltage sensitive $\text{Na}^+$ channels to open. This massive influx of positive ions triggers an action potential in the innervated muscle fiber. This impulse is rapidly conducted throughout the muscle fiber via the T-tubules. When the action potential arrives at a triad, it triggers the release of $\text{Ca}^{++}$ sequestered in the sarcoplasmic reticulum. Here, free $\text{Ca}^{++}$ ions combine with the troponin molecules of thin filaments. In a resting muscle fiber, troponin prevents the myosin molecules of the thick filaments from interacting with the actin molecules of thin filaments. When $\text{Ca}^{++}$ is present, myosin interacts with actin and “ratchets” the thin filaments toward the center of each sarcomere. This shortens the sarcomere and thereby shortens the myofibrils and the muscle fibers they compose (see FIG. 4). If a sufficient number of fibers shorten, the muscle contracts.
FIGURE 4  (From Thibodeau, 1990)
Controlling the Strength of Skeletal Muscle Contraction:

While the individual muscle fibers obey an all-or-none principle (they either fully contract or do not contract at all), the entire skeletal muscle can contract with varying degrees of strength (think of picking up a feather versus a bowling ball). This is effectively termed the **graded strength principle** and several factors dictate how forcefully a given muscle will contract (see FIG 5):

![FIGURE 5](From Thibodeau, 1990)

The strength of a skeletal muscle contraction bears a direct relationship to the initial length of its fibers, their metabolic condition, and the number of them contracting. If a muscle is moderately stretched at the moment when contraction begins, the force of its contraction increases. ... Outstanding among metabolic conditions that influence contraction are oxygen and food supply [glucose].

With adequate amounts of these essentials a muscle can contract with greater force than is possible with deficient amounts. The greater the number of muscle fibers contracting simultaneously, the stronger the contraction of a muscle. How large this number is depends on how many motor units are activated, and this in turn depends on the intensity and frequency of the stimulation [See Fig. 6]. In general, the more intense and more frequent a stimulus, the more motor units and therefore the more fibers are activated and the stronger the contraction.

Another factor that influences the force of contraction is the size of the load imposed on the muscle. Within certain limits, the heavier the load, the stronger the contraction. (Thibodeau, 1990)
Types of Skeletal Muscle Contraction:

Skeletal muscle contractions can be tonic, isotonic, isometric, twitch, and tetanic.

A *tonic contraction* is a continual, partial contraction. At any one moment a small number of total fibers in a muscle contract, producing a tautness of the muscle rather than a recognizable contraction and movement. Tonic contraction...is particularly important for maintaining posture.

An *isotonic contraction* is a contraction in which the tone or tension within a muscle remains the same but the length of the muscle changes. It shortens, producing movement.

An *isometric contraction* is a contraction in which muscle length remains the same but in which muscle tension increases.

A *twitch contraction* is a quick, jerky contraction in response to a single stimulus.

A *tetanic contraction* is a more sustained contraction than a twitch. It is produced by a series of stimuli bombarding the muscle in rapid succession. (Thibodeau, 1990)
II. Experimental Setup

The data acquisition hardware you will use in this experiment is identical to the equipment used in the modified Loewi’s experiment. It consists of the MacLab/400 unit, ETH-250 bridge/bioamplifier, and Macintosh PowerPC. A new component of hardware is the force transducer which measures the tension that the muscle produces when it contracts isometrically. MacLab’s Chart program is also used.

III. Method

Setting up the Hardware:

4) Check to ensure that a BNC cable is connecting the output of the ETH-250 to the CH1 input of the MacLab/400 unit.
5) Check to see that the force transducer is connected to Channel 1 on the back of the ETH-250 amplifier.
6) Set the Mode to bridge, Gain to 100, and Filter to W.B. (wideband).
7) Using two BNC cables, connect one end of each cable to the prongs of the stimulating electrode. Connect the other end of each BNC cable to the Output (+) and (-) on the MacLab/400 unit. (It does not matter which prong of the stimulating electrode is connected to the (+) and (-) terminals on the MacLab unit.)
8) Attach the stimulating electrode to one of the ring stands so that the electrodes are parallel to the tabletop.
9) Attach the force transducer and femur clamp to the other ring stand so that the dissected muscle can be suspended between the two with the force transducer on the top.
10) Tie one end of a length of suture thread to a fish hook, and the other end to the arm of the force transducer.

Setting up the Software:

1) Double click on the Chart icon in the MacLab folder. After some initialization, the main chart window is shown. By default, Chart displays 8 channels. To the right of the display window for a given channel are some controls for that channel. To the right of channel 2, click and hold on the Channel 2 control and select “Turn channel off…” from the pop-up menu. Repeat this process for channels 3 & 4 (channels 5-8 are off by default).
2) Click and hold on the double line separating the display window for channel 1 and channel 2. Drag this line to the bottom of the screen so that the display for channel 1 occupies the entire screen.
3) To the right of the Channel 1 window, click and hold on the Channel 1 control, and select Input Amplifier from the pop up menu. Make sure that both the Positive and Negative boxes are selected. Gently tap the force transducer and note that a response is generated on the display.
4) Decrease the Range to 1V (if the muscle’s response is ever too small or too large to be plotted on the screen, stop the chart recorder by pressing STOP in the lower right hand corner of the screen and adjust the range).
5) Adjust the Input Offset knob on the ETH-250 to center the signal in the display. After you have done this, click OK.
6) Adjust the speed to 500 msec which will appear as 40 samples/sec in the sample speed box.

The Dissection:

Your TA will provide each table with the hind limb of a frog, the gastrocnemius ("calf") muscle should be dissected from the limb in the following manner:

1) Remove the skin from the limb by making a longitudinal incision down the length of the leg, being extremely careful not to cut the underlying musculature. Cut the locations where the skin is attached to the underlying musculature (make sure that ALL attachments are cut otherwise the skin cannot be easily removed).
2) Holding the frog firmly, grasp the cut skin with forceps and pull downward—peeling the skin from the hindlimb in one piece.
3) Moisten the muscles with frog Ringer’s solution. (It is necessary to keep the muscles moist at all times!)
4) Identify the gastrocnemius muscle. Push a squeezed pair of forceps underneath the Achilles tendon, grab a piece of suture thread, and pull it back through under the tendon. Tie the suture tightly around the tendon to keep it from fraying during the course of the experiment.
5) Using a sharp scalpel blade, cut the Achilles tendon distal to the suture (leave as much tendon attached to the muscle as possible, since it will be used to attach the muscle to the transducer).
6) Pull the tendon away from the tibia/fibula to free the distal end of the gastrocnemius muscle.
7) Dissect away the muscles of the upper leg and expose the femur. Using a pair of bone shears, cut the femur above the origin of the gastrocnemius.
8) Continue using the bone shears and cut away the tibia/fibula.

Attaching the Muscle to the Experimental Apparatus:

1) Insert the fishhook into the Achilles tendon (attaching the distal end of the muscle to the force transducer).
2) Clamp the femur into the femur clamp (thereby attaching the proximal end of the muscle to the stationary femur clamp).
3) Adjust the height of the force transducer until the slack is JUST taken out of the muscle (a loose muscle will confound your results, as will a muscle that is stretched too tightly).
4) Place the two prongs of the stimulating electrode firmly against the muscle (one potential source of experimental error occurs when the tips of the stimulating electrode lose contact with the muscle during stimulation, when stimulating the muscle for the first few trials, have someone observe the muscle to ensure that contact is not broken during the course of the stimulation).
5) Ensure yourself that you are satisfied with your preparation—any modifications beyond this point may confound your data (ASK YOUR TA if you feel like you need to modify your experimental setup).

IV: Experimental Conditions

Before beginning each and every condition, thoroughly wet the muscle with frog Ringer’s solution. Do not wet the muscle during any condition (it could potentially confound your results).

Condition 1: Determining the Stimulus-Response Relationship

1) Wet the muscle with frog Ringer’s solution.
2) Open the Input Amplifier in the Channel 1 control window and adjust the Input Offset so that the trace is positioned at zero (be as precise as you can).
3) Under the Set-up menu, click on Stimulator.
   a) Click “on” to activate the stimulator.
   b) Set the number of pulses to 1
   c) Set the pulse duration to 5 msec.
   d) Set the amplitude range to 10 volts
   e) Set the actual amplitude to 0 volts.
   f) Close the stimulator window.
4) Under Set-up click on Stimulator panel and position the newly created Stimulator window in a location on the screen that doesn’t obscure anything of importance.
   a) This panel displays the stimulus parameters in a window that makes it very convenient to make adjustments to the stimulus attributes (i.e. amplitude).
   b) You will also use this panel to apply stimuli to the gastrocnemius.
5) Click on Start in the lower right hand corner of the screen to begin the chart recorder.
6) Click the Stimulate button in the Stimulator window to apply the stimulus to the muscle.
7) Once the stimulus has been delivered, press Stop in the lower right hand corner of the screen.
8) Using the cursor, record the muscle tension produced by the stimulus (use the blowup measurement window for precise measurements).
9) Adjust the stimulus amplitude in the stimulator window by 0.5V and repeat steps 5-8.
10) Repeat this entire process (steps 5-9) until three consecutive stimuli fail to further increase the force of twitch contraction.
11) Using a computer graphing package (e.g. Excel), plot muscle tension versus stimulus intensity (be sure to label your axes).
Condition 2: Creating a Tetanic Contraction

In condition 1, you applied single stimulus shocks to produce twitch contractions. Now you will adjust the **Frequency** of the stimulus to deliver repeated shocks to the muscle. This will allow you to ultimately create a tetanic contraction.

1) Wet the muscle with frog Ringer’s solution.
2) Open the **Input Amplifier** in the **Channel 1** control window and adjust the **Input Offset** so that the trace is positioned at zero (be as precise as you can).
3) Under the **Set-up** menu, click on **Stimulator**.

**II.** Set the number of pulses to **continuous** to calibrate the software to deliver continuous stimuli to the muscle.

**III.** Set the stimulus amplitude to the value that produced maximal twitch contraction in condition 1.

**IV.** Set the range to 20 Hz.

**V.** Set the frequency to 2 Hz.

**VI.** Close the stimulator window.

**IV.** The **Stimulator** panel should now display **on** and **off** buttons in place of the **Stimulate** button. Click the **Off** button so that stimuli are not being delivered to the muscle.

5) Press the **Start** button in the lower right hand corner of the screen to start the chart recorder.
6) After a few seconds of baseline data have been recorded, click the **On** button in the **Stimulator** window.
7) When muscle tension becomes constant, press the **Stop** button (A couple of seconds should be adequate at each frequency).
8) Using the cursor, record the muscle tension produced by the stimulus (use the measurement window in the upper right section of the screen for precise measurements).

8) Adjust the **Frequency** in the **Stimulator** window to 4 Hz and repeat steps 5-7.

9) Continue to repeat steps 5-8 while successively adjusting the frequency to the following approximate values (8 Hz, 15 Hz, and 30 Hz). Thus, at the end of this condition you will have measured muscle tension in response to stimulus frequencies of 2 Hz, 4 Hz, 8 Hz, 15 Hz, and 30 Hz. **Note:** you will have to increase the range for the 30 Hz frequency.

10) **Calculate tetanus-tension ratios for each frequency and plot the results using a computer graphing package.**

   a) Tetanus-twitch tension ratios = \[
   \frac{\text{Maximum tetanic tension}}{\text{Maximum twitch tension}}
   \]
Condition 3: Investigating the Length-Tension Relationship

In this condition, you will investigate the effect of muscle length on contraction strength. To do this you will apply a stimulus and record the muscle tension produced when the muscle is at different lengths.

1) Wet the muscle with frog Ringer’s solution.
2) Adjust the height of the force transducer so that the muscle is just slack.
3) Using points on the muscle that you can remember (and are highly repeatable), measure the length of the muscle to the nearest mm.
4) Under the Set-up menu, click on Stimulator.
   a) Set the number of pulses to 1
   b) Set the pulse duration to 5 msec.
   c) Set the amplitude range to 10 volts
   d) Set the actual amplitude to the value that produced maximal twitch contraction in condition 1.
   e) Close the stimulator window.
5) Open the Input Amplifier in the Channel 1 control window and adjust the Input Offset so that the trace is positioned at zero (be as precise as you can).
6) Stimulate the muscle and record the tension produced.
7) Raise the force transducer until the resting length of the muscle has been lengthened 2 mm.
8) Readjust the Input Offset so that the trace is positioned at zero (again, be very precise).
9) Stimulate and record the tension produced.
10) Repeat this process (steps 7-9) until active tension peaks and clearly begins to decline.
11) Using a computer graphing package, plot tension versus length (don’t forget units).
V: Lab Report

Your lab report should follow the abstract-introduction-methods-results-discussion format introduced previously.

The introduction should be comprised of general information about the muscular system and neuromuscular modulation. As it develops, the introduction should propose the experimental hypothesis and introduce the experiments designed to test it. Remember, the introduction is used to capture a reader’s attention, so try to formulate it so that it provides information relevant to a wide readership (convince the reader that this is an exciting study).

The methods should be relatively straightforward. You need to describe the experimental setup, including general information about the hardware and software. You also need to describe each of the experimental conditions in sufficient detail that a reader unfamiliar with the experiment could replicate it on his/her own. Remember, no results should be included in the methods section.

The results will contain all of the tables, charts, and figures required for each condition (see the last step in every condition for specific instructions). The body of your results section should include a written description of the main point/trends of the figure (make sure that you refer your readers to the figures, tables, and charts in the appropriate location in the written description).

The discussion section should compare your results with the results you had expected to obtain given previous research and general information about the muscular system. Any discrepancies should be mentioned, and you should theorize why they occurred. Additionally, any unusual results should be explained in this section.

References


Neuroscience Methods
VII. Histology I: An Introduction

Materials
- Fixed rat brain
- Cryostat, microtome, or other means of sectioning
- Subbed microscope slides
- Materials for cresyl violet staining protocol (see methods section)
  - absolute ethyl alcohol
  - distilled water
  - cresyl violet acetate (e.g. sigma #C5042)
  - glacial acetic acid
  - clearing agent (e.g. histoclear - Baxter #C4200-1)
- Staining dishes and slide racks
- Mounting media (e.g. Permount)
- Q-tips
- Coverslips
- Low power light microscope or a light box and magnifier
- Stereotaxic atlas, such as Paxinos and Watson [1]

Purpose
- To become familiar with basic histological technique. To use staining techniques in combination with a stereotaxic atlas to identify anatomical structures in sectioned brain tissue.

Background

Histology refers to the microscopic study of tissue. The visualization of the brain’s cytoarchitecture is an important complement to the studies of function and gross anatomy performed in other labs. It is used in a number of areas essential to neuroscience research, such as identifying nuclei affected by a lesion and verifying the placement of a subcortical electrode.

Histology also refers to the techniques used to prepare tissue for microscopic study. This includes not only staining tissue for light and electron microscopy, but also more advanced techniques such as tracing fiber tracts, identifying receptor types present in a given brain region, and mapping the distribution of the expression of a particular gene. These latter techniques are more properly described as histochemistry, which is the (art and) science of localizing the various chemical constituents of cells. This is an exciting field that makes use of methods from areas as diverse as biochemistry, molecular genetics and image processing.

Contributed by M. Jones, Spring 1996
In this lab, we will prepare sections of brain tissue for viewing using a light microscope. In order to be visualized, the tissue must first be fixed and stained. Fixing prevents enzymatic and other postmortem changes that degrade the tissue. Typically, the animal containing the brain of interest is sacrificed and perfused, i.e. the blood is drained from the body and a fixative solution is pumped into the vascular system. Removing the blood improves the results of staining, and the fixative preserves and usually hardens the brain so that it can be sectioned (cut into thin slices) without tearing. The sections, cut sufficiently thin to view using a light microscope, are then stained to bring out cellular details that would otherwise be difficult to observe.

There is a wide variety of staining techniques. In neuroscience, perhaps the most familiar is Golgi staining. For reasons still unknown, this technique stains only a few cell bodies in the tissue in their entirety, thereby allowing a detailed visualization of individual neurons (note a Golgi stain can take several months to complete!). Other techniques include myelin stains for visualizing fiber bundles, degenerating-axon stain which specifically stains dying axons, and several techniques for cell body (Nissl) staining.

In this lab we will be using cresyl violet, a Nissl stain that colors cell bodies a brilliant violet. Nissl is a term used by classical cytologists for the endoplasmic reticulum. Since all cells contain ER, cresyl violet will stain both neurons and glia. An outline of the procedure consists of three steps:

1. Sectioning the brain.
2. Placing the brain sections on slides and applying the stain.
3. Viewing the stained sections in conjunction with a brain atlas.

Routine staining for light microscopy typically requires sections of about 50µm in thickness. While crude sections may be prepared with a razor blade and a steady hand, sectioning is usually performed using a cryostat, microtome, or vibratome. Each of these provide some means of securely holding the tissue and passing a razor or special knife through it at a precise angle. With each pass of the blade the tissue is advanced a specified distance such that uniformly thick sections are obtained. Because even fixed tissue can be difficult to section cleanly, the tissue is usually frozen (cryostat) or embedded in paraffin (wax) when using a microtome. The vibratome uses a vibrating blade so freezing or embedding is usually not required.

The staining procedure consists of sequentially dipping the slides in about a dozen different solutions for specified amounts of time (see next page). Although the sequence of solutions may appear to be arbitrary, it is designed to accomplish the following:

1. An initial alcohol soak removes lipids (fats) and fixation chemicals from the tissue.
2. The sections are submerged in stain.
3. The tissue is dehydrated (water is driven out) by a series of alcohol baths.
4. The unstained parts of the tissue are made transparent by a clearing agent.

The series of decreasing alcohol baths between the initial soak and the stain are necessary because the stain is water-based (i.e. made up in water) and so the tissue would not stain well if placed directly in the stain following the initial alcohol soak because alcohol and
water are not miscible. Also note that an acidic bath is included in the dehydration sequence. This serves to remove excess stain from the tissue.

Collections of stained cell bodies form structures that can be identified with the aid of a stereotaxic atlas. An atlas is a collection of pictures of brain sections with accompanying diagrams of structures that can be identified in each section. One such figure is provided in Appendix A. This plate includes an indication of the section’s position, a view of the section itself, and a sketch of the major structures which can be identified. While this plate show only a coronal section, most atlases also include sections along sagittal and/or horizontal planes, and often include coordinate axes so that identified structures may be precisely located using a stereotaxic apparatus.
Method

1. Your TA will provide you with a slide containing 2-5 sections. Carefully blot away any excess moisture from around the tissue. Allow to dry sufficiently (5-15 min) so the sections do not fall off during staining.

2. Insert your slide into one of the carriers provided. Pass the carrier through the following sequence of baths, observing the times indicated:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% ETOH</td>
<td>15 min</td>
</tr>
<tr>
<td>70% ETOH</td>
<td>1 min</td>
</tr>
<tr>
<td>50% ETOH</td>
<td>1 min</td>
</tr>
<tr>
<td>DH2O</td>
<td>2 min</td>
</tr>
<tr>
<td>DH2O</td>
<td>1 min</td>
</tr>
<tr>
<td>Cresyl Violet Stain(^7)</td>
<td>2 min.</td>
</tr>
<tr>
<td>DH2O</td>
<td>1 min</td>
</tr>
<tr>
<td>50% ETOH</td>
<td>1 min</td>
</tr>
<tr>
<td>70% acid ETOH(^8)</td>
<td>2 min.</td>
</tr>
<tr>
<td>95% ETOH</td>
<td>2 min</td>
</tr>
<tr>
<td>95% ETOH</td>
<td>a few dips</td>
</tr>
<tr>
<td>100% ETOH</td>
<td>1 min</td>
</tr>
<tr>
<td>Histoclear(^9)</td>
<td>5 min</td>
</tr>
</tbody>
</table>

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\(^6\) ETOH = ethyl alcohol; DH2O = distilled water; all solutions 200 ml

\(^7\) add 1.25g cresyl violet acetate and 0.75 ml glacial acetic acid to 250 ml warm DH2O, cool and filter

\(^8\) 2 ml glacial acetic acid in 200 ml 70% ETOH

\(^9\) Some people find histoclear vapor to be irritating. You may want to wear a mask during this procedure.
After finishing this “gauntlet”, let the carrier drain on a paper towel for about 30 seconds, then remove the slides from the carrier. Wipe off excess clearing agent from around the tissue. Using the shaft of a Q-tip, immediately apply a small drop of mounting media to each section and slowly lower a coverslip onto the slide. Gently press out any air bubbles that may have formed. Note that mounting media tends to get everywhere except where you want it to - wear gloves and be as careful as possible.

Viewing

Using either a light box and a magnifier or a light microscope on low power, examine your stained sections. Cell bodies will appear as violet to dark blue. Fiber tracts will remain white or light colored. Under higher magnification, some of the subcellular organelles may be visible (usually the cell nucleus).

Compare your stained sections to the illustrations in the stereotaxic atlas. Note that the anatomy of the rat brain (especially the hippocampal structures) differs greatly from that of the human brain. Pick your best section and make a rough sketch. Identify at least 5 structures using the atlas, including at least one nucleus and one fiber tract. What is the approximate position along the rostral/caudal axis of your section? Using the atlas, order all the slides in your group starting with the most rostral and ending with the most caudal. Are there sections missing in your collection?
Suggestions for Further Study

Many of the histology books at Norlin library are somewhat dated. While the reference section is your best bet for standard textbooks, for hands-on information, try Palkovits and Brownstein [2], Davenport [3], Humason [4], Mesula [5] and Luna [6]. These contain excellent tips on technique and include scores of recipes for various protocols. For books focusing more on the chemistry of staining, look around in the QH320’s. Here you will also find books on light and electron microscopy, and techniques such as freeze-fracturing. For a fascinating look at “applied histology”, try the Center for Human Simulation web site (http://www.uchsc.edu/sm/chs). The people at CHS have fixed an entire human body, sectioned at regular intervals, then by combining the cross sections of organs in sequential sections, reconstructed the 3D geometry of several organ systems!

Study Questions

1. How does “histology” differ from “histochemistry”?

2. What does cresyl violet stain? Why does removing blood from the tissue improve the results of staining (be specific)?

3. The following are some problems that can occur during staining. Give a probable cause of each.

   a) One or more chemicals used in fixation reacted with the stain, ruining the section.
   b) Water remained in the section, resulting in a foggy slide.
   c) The finished section was too light (list two corrections).

4. What are some uses of stained sections?

5. Staining can be classified as progressive, in which the section is removed from the stain once sufficient stain has been absorbed, and regressive in which the section is over stained and the excess stain removed using a solvent. Which type of staining is the cresyl violet protocol?
References


Appendix A - Sample Plate from Palkovits and Brownstein [2]
ANSWERS

Question 1)

_Histology_ refers to the microscopic study of tissue and the techniques used to _prepare_ tissue for microscopic study.

_Histochemistry_, on the other hand, is the science of localizing the various chemical constituents of cells. This includes identifying receptor types present in a given brain region and mapping the distribution of the expression of a particular gene.

Question 2)

_Cresyl violet_ is a Nissl stain, and Nissl is a term used by classical cytologists for the endoplasmic reticulum. Therefore, _cresyl violet_ stains the cell’s endoplasmic reticulum.

Because blood is composed of blood cells and is responsible for the transport of myriad other cells, and because these cells all contain Nissl substance (endoplasmic reticulum) any remaining blood in the tissue will be deeply stained by cresyl violet, and the “important” features of the structure will be obscured by this artifactual staining.

Question 3)

a) One or more chemicals used in fixation reacted with the stain, ruining the section.
   The initial alcohol soak was not thorough enough to remove all the fixation chemicals.

b) Water remained in the section, resulting in a foggy slide.
   Water is removed from a sample prior to staining by dehydration: “running” the sample through a series of graded alcohol baths of increasing concentration. If water remained in a sample, resulting in a foggy slide, it would be highly likely that the sample was passed too quickly through the alcohol baths, or the samples were not passed through the highest concentration alcohol bath (100% alcohol). Stopping prior to the 100% alcohol bath would leave 5% water in the sample if the subsequent bath was 95% alcohol.

c) The finished section was too light (list two corrections).
   If a resulting section is too light, then either the staining time was too short, or too much stain was removed during the dehydration and clearing stages of the protocol.

Question 4)

Stained sections are used to verify the location of an electrode that had been lowered into the brain using stereotaxic coordinates. Stained section also reveal the cellular morphology of different areas of the brain. Stained sections are also used in the creation of stereotaxic atlases, and are used to identify the boundaries of different nuclei.
Question 5)

Cresyl violet is a *regressive stain*. If the samples were directly removed from the staining bath, dehydrated, and cover slipped, it would be impossible to discern any cellular morphology. The clearing agent is necessary to remove excess stain from non-nissl containing structures (i.e. fibers of passage). The clearing agent is responsible for the staining “regression.”
VIII. Histology II

Materials:
Nissl stained rat brain sections (see previous histology lab)
Prepared slides of human nervous tissues (Carolina Biological - Slide Set No. 720)
Microscopes

Purpose:
By the end of the lab, using a light microscope, you should be able to 1) distinguish neurons from glial cells 2) identify the different types of glia and 3) identify prominent subcellular organelles. In addition, you should be able to identify any of the slides from the human nervous tissue collection, and be able to discuss the salient features of each of these preparations.

I. Background

Although many advanced techniques in histology and histochemistry are used in neuroscience research, basic light microscopy still occupies a central role in the laboratory. This discussion extends the concepts introduced in the previous histology lab to include the use of the light microscope. We will examine Nissl stained sections at higher magnification in order to identify neurons and glia. In addition, we will examine slides from the Carolina Biological collection, which includes sections from human cortex, brainstem, cerebellum, and spinal cord.

II. Use of the Light Microscope

Since you will be working independently, it is helpful to have an overview of the proper use of the microscopes that we have available in the lab. This will allow you to concentrate on anatomy during the lab period rather than struggling with the equipment. This also prevents damage to the microscopes and the slides from improper use. Although use of the light microscope is straightforward, there are some subtleties which can be frustrating. Please read this section thoroughly before coming to lab, so that you will be prepared to start working when lab begins.

The figure at the right (Junqueira, et. al. 1983) shows the important controls of the light microscope, which include the condenser, light filter, stage position adjustment, and focus adjustment knob. This microscope differs slightly from those you will be using, in that it only has a single eyepiece, two object lenses, and the light source is located in the base. The microscopes we have are binocular (two eyepieces), have four object lenses (4x, 10x, 40x, and 100x) and the light source attaches to the base directly underneath the condenser.

1 Contributed by M. Jones, Spring 1997.
Revised Fall 2000, C.P. Stark.
Lighting adjustment is usually the most confusing part of using a microscope. The microscopes in the lab have separate power supplies for the light source, with an on/off switch and a dial adjustment for brightness. The dial should be set about halfway. There are three other lighting adjustments you should be aware of. There is a light filter on the bottom of the condenser that can be swing out of the path of light source. Keep it in place. There is a lever on the front of the condenser that adjusts the aperture of the condenser (like a camera). Adjust it so that it is fully open. Finally, there is a knob on the side of the condenser which raises and lowers the condenser. When using the lowest power objective, lower the condenser as far as possible. Otherwise raise the condenser until it just contacts the stage. The small knobs on the front of the condenser simply secure it to the scope and are not used for light adjustment (please don’t disassemble the microscopes!)

To summarize, if the section appears too dark, try the following (in order): raise the condenser, makes sure the aperture is fully open, and increase brightness on the power supply. If the section appears too light, or only a portion of the visual field is illuminated: lower the condenser, tighten the aperture, and decrease brightness on the power supply. Always make sure the light filter is engaged.

To view a slide, lower the stage using the coarse focus adjustment, rotate the lowest power (i.e. shortest) objective into the center position, and place the slide on the stage with the cover slip facing up. If the slide is inserted coverslip–down, it may not be possible to bring the section into focus. The slide is typically held in place with a spring-loaded clip. This allows the slide to be moved around using the stage position adjustment. There are two controls for this: one moves the slide left and right, the other moves the slide forward and back. Once the slide is secured on the stage, look at the stage and slowly raise it using the coarse adjustment until the slide almost contacts the bottom of the objective. Look though the eyepieces, and lower the stage using the coarse and fine adjustment until the section is in focus. Let’s make absolutely clear the first commandment of light microscopy which we have hinted at in this procedure:

**Never raise the stage while you are looking through the eyepieces.**

You should only use the coarse adjustment to raise the stage only if you are looking at the stage. Otherwise, there is a good chance that you will drill the objective through the slide. The slide will break, the objective will be damaged, and you will be a couple hundred dollars poorer.

Changing the view magnification is a simple question of rotating a new objective into place. Before doing this, check that the objective will not contact the slide as it is rotated into place. If the objectives are par-focal (which they should be, but some we have are not), you should not have to move the stage, and the image should remain in focus (to within fine adjustment). If there is not enough room, use the coarse focus to lower the stage, rotate the objective, then look at the stage and slowly raise the stage until it almost contacts the slide. Return to viewing and lower the stage using coarse and fine adjustment until the section is in focus. Note the highest power objective (usually 100x) cannot be used without immersing the specimen in special oil which corrects the optics for high power magnification. To recap:

**Never raise the stage while you are looking through the eyepieces.**

---

2 Coarse and fine focus is controlled by a single knob. Fine adjustments are made by small movement of the focusing knob, whereas larger rotations engage the coarse focus. On some microscopes the coarse adjustment moves the stage and on others it moves the objectives. Substitute the phrase “lower the objectives” for the phrase “raise the stage” where appropriate.
III. Light Microscopy of Rat Brain Nissl Sections

In the previous histology lab, we used a low power dissecting microscope in conjunction with a stereotaxic atlas to identify nuclei, fiber paths and other structures in Nissl stained sections of rat brain. In this lab we will study individual cells. We will identify neurons and glia, and distinguish the different types of glial cells. Higher magnification will also allow subcellular organelles to be identified, such as the cell nucleus, nucleoli, endoplasmic reticulum and golgi complex. Read over the following, then spend some time with a good Nissl section and try to identify the features of the neurons and glia that are described below.

As discussed previously, Nissl substance is what classical anatomists called the endoplasmic reticulum. Exposing the tissue to a stain which attaches to the ER allows us to differentiate cell bodies from fiber paths, and from the surrounding extracellular matrix. Somewhat surprisingly, Nissl staining also allows us to differentiate neurons from glia, if we know what to look for. It turns out that neurons and glia contain different amounts of endoplasmic reticulum, so that when viewed under the microscope, anything with a cell body (or “perikaryon”), especially if any processes are visible, are neurons. Glial cells don’t have much endoplasmic reticulum, so only their nuclei stain. The figure to the right illustrates this nicely (taken from Nauta, 1986). Here, the cell bodies and processes of neurons (N) can be easily distinguished from the glial cells (O, A, and M). The dark grains in the neurons are clumps of Nissl substance. The light regions in the center of the neurons are nuclei. Note that they occupy a large portion of the cell body and are rather pale. The large nucleus reflects the amount of protein synthesis required by neurons. Neurons do not divide, so their genetic material is not condensed (i.e. the chromatin is extended). This is the reason for the pale appearance of nucleus. The dark spots in the nuclei are the nucleoli, which are sites where ribosomes are produced.

All of the neuronal processes visible in the above figure are dendrites. A dendrite from the neuron at lower left is labeled with an arrow (“<”). The elongated dark clumps are Nissl substance. Only the proximal portion of a dendrite is visible - the process is soon obscured by the (unstained) cell bodies of glia. Axons are pretty tough to see, simply because they are so thin. It so happens that the axon hillock does not contain Nissl substance. Therefore, the easiest way to identify an axon is by first finding an axon hillock, which will appear as a light cone–shaped process free of Nissl grains. If you examine the distal end of the process, you can sometimes make out the thin trace of an axon.

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3 Do not confuse “nucleus” (for example: red nucleus) with “cell nucleus”. The former is a collection of cells in the central nervous system that collectively perform a specific function, whereas the latter is a subcellular organelle where genetic material is found.
There are three types of glial cells identifiable in the above figure. The curved row of four round dark nuclei are oligodendrocytes (O). To the left of these is a larger, irregularly shaped, and lighter staining astrocyte nucleus (A). Finally, abutting the right end of the row of oligodendrocytes is a small angular pellet, which is the nucleus of a microglial cell. Again note: no perikaryon or processes are visible in glia-nuclei only.

The nuclei of glial cells are darker than those of neurons because the chromatin in glia is condensed (glia maintain the capacity for cell division). The nice round shape of oligodendrocyte nuclei is pretty dependable, however the shape of astrocytes varies somewhat, as does their color and size. Sometimes the most reliable way to identify a glial cell is its location in the tissue. Oligodendrocytes are responsible for myelinization, and appear as rows of dark spots in fiber tracts (see the corpus callosum, for example). Fibrous astrocytes are also located in white matter, whereas protoplasmic astrocytes are found in gray matter. As discussed below, endothelial cells are typically associated with blood vessels; ependymal cells (not shown) line the ventricles and central canal. Don’t confuse ependyma with cells of the choroid plexus. The latter appear small and round, and are often found in coiled strips in the ventricles themselves.

A final component which can be identified in stained sections are capillaries. These usually appear as a hole or cylinder in the tissue in which cells (and everything else) is conspicuously absent. A capillary can be see in the figure above, labeled “C”. Here, two endothelial cells lining the walls of the capillary appear as dark crescents. Sometimes individual erythrocytes (red blood cells) can be identified in the capillary, especially when using fast blue staining, which gives blood cells a greenish tint (see below).

### IV. Beyond Nissl Staining

Nissl staining is just one of dozens of biological staining techniques used in neuroscience research. Some other common techniques are briefly described below. As you will encounter these stains in the Carolina Biological slide collection, these descriptions will help you understand what you are looking at when you view their sections.

1) *Hematoxylin and Eosin* (“H&E”). This is a commonly used combination of dyes that stains cell nuclei blue and cell cytoplasm (and just about everything else) an ugly pink. H&E is a favorite of the Carolina Biological folks; if the slide tag doesn’t list the staining protocol, it’s probably H&E. See the entire first chapter in Ham for an in–depth discussion of what exactly H&E stains and why.

2) *Fast Blue* (sometimes called *Luxol Blue*). This is a myelin stain. It will stain fiber paths in both the central nervous system and the periphery, provided the axons are myelinated. Sometimes fast blue is combined (“counterstained”) with a Nissl stain so that both fiber paths and cell bodies can be identified. One oddity about fast blue is that it tends to turn blood cells green, which can help you identify blood vessels in a section. See Ham for why this happens.

3) *Silver* (reduced silver, actually, to distinguish it from the silver method used in Golgi stains). Silver stains components of the cytoskeleton, specifically the microtubules and neurofilaments. This is not so interesting in cross–section, but in other views, silver staining allows you to follow the course of axons. Cell bodies and the rest of the tissue appear as dark brown.

4) *Neutral Red* This is another Nissl Stain. It is often used instead of cresyl violet for counterstaining fast blue sections because the red stands out against the blue better than violet.

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4 Behold, the blood–brain barrier!
V. The Carolina Biological Human Nervous Tissue Collection

The following narrative describes some of the slides of human tissue available from Carolina Biological. The entire collection consists of about 25 slides of human cortex, brainstem, cerebellum, and spinal cord, from which we’ve picked out a 10 or so and given a brief description, and a synopsis of features you should be able to identify. Keep in mind that each box of slides is slightly different, and that some features described here may be missing on a given sections. Also, keep in mind that most human tissue don’t come from perfused humans, so the tissue preparation is often not as good as what can be done with rat brains. Alcohol is commonly used as a fixative, which often leads to shrinkage (have a look at the spaces around the motor neurons in the spinal sections for a good example of this).

The narratives are listed by slide label, in no particular order. Locate each slide in your collection, read over the description, and spend some time viewing the section.

1) Cerebellum, Fast Blue (sec. H 6816)

This slide, a sagittal section prepared with fast blue and counterstained with H&E, clearly shows the three layered organization of the cerebellum. A similar preparation is shown below at right (Martin, 1996). The outer or molecular layer is stained pink and contains basket cells, stellate cells, and parallel fibers. The granular layer is dark blue, and contains granule and golgi cells. A layer of Purkinje cells lies between the molecular and granular layers, and are also stained dark blue. Axons exit beneath the granular layer and form the white matter of the cerebellum, stained light blue in the section.

The Nissl counterstaining in this slide really does not do justice to the Purkinje cell. A Golgi stain of a Purkinje cell showing the extensive arborization is shown at upper left. These cells make as many as 100,000 synaptic contacts! The highly organized and impressive cytoarchitecture that the cerebellum exhibits at all levels reflect its specific functional

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5 Refer to your textbook for a discussion of the structure of the cerebellum.
requirements. Although the cerebellum is known to play a role in motor control, how it actually works is still not known.

2) Cerebellum, Silver (sec. H 6815)

This is an identical section to the fast blue slide, except stained with silver. Note that the white matter stains dark brown in this section, and appears fibrous. Note the thin black lines coursing though the Purkinje layer (at 40X). These are axons from basket cells. Larger brown branches or clumps in the molecular layer are blood vessels. The black “specks” in the granular layer are probably glomeruli. Finally, note the collection of smaller black specks at the interface between the Purkinje and granular layers. These are axon terminals of basket cells wrapping around the cell body of Purkinje neurons, and are shown (open arrow) at much higher magnification in the figure below (taken from Palay and Chan-Palay, 1974). The Purkinje cells are indicated by dotted lines:

![Image of cerebellum with silver staining]

3) Spinal Cord, Lumbar Region, Cross-Section (sec. H 6850)

This is a fast blue section counterstained with H&E. The lumbar region of the spinal cord is where the legs plug in – note the huge motor neurons located in the ventral horn (at 40X). These cells are prime hunting ground for axon hillocks and axons. Note the spaces around the cells - this is shrinkage due to alcohol fixation. You can also make out what's left of the dorsal root ganglia, and some local fiber tracts. You might be able to see ependymal cells lining the central canal, but in a lot of these sections the central canal is not very prominent.

4) Basal Ganglion (sec. H 6808)

This is a myelin stain, counterstained with H&E, of a region of the basal ganglia. The large blue area is the internal capsule. Note the cell bridges that cross the internal capsule (at 10X and 40X). Similar bridges were evident in the sheep brain coronal sections. This is why the putamen and caudate are called the “striatum”, which is Latin for “striped”.

![Image of basal ganglion with myelin staining]
5) Substantia Nigra (sec. H 6823)

This is another H&E section. Of particular interest is the collection of large, darkly staining cells. These are the dopamine–producing cells of the substantia nigra pars compacta and are the cells that degenerate in Parkinson’s disease. Note the dense vascularization in this region (the capillaries appear as pink clumps). No neuron is far from a blood vessel, and in some instances a capillary is “attached” to the cell. This was noted by Scheibel and Tomiyasu in a 1980 article in Experimental Neurology (see references), however the implications are not clear.

6) Pons (sec. H 6825)

Absolutely packed with axons, this section should give you a feel for the tremendous density of fiber paths in the pons. This is the site where ascending and descending fibers send collaterals to the cerebellum. H&E stain.

Medulla Fast Blue, Medulla Silver (sec. H 6820/ 6821/6822)

Between the three medulla slides, you should be able to identify the inferior olives (light purple at 2.5X), pyramids, and the decussation of the medial lemniscus. The level of your section may vary, but the following figure should be of assistance (from Heimer, 1995):

The decussation of the medial lemniscus is most easily identified in the silver stain - it looks like a small ribcage in the center of the section. Sometimes segments of choroid plexus from the 4th ventricle can be found (try the H&E section). If you have a rat coronal section that contains choroid plexus, a comparison of the two will show that they are almost identical.
Acknowledgements

We would like to thank Dr. Eva Fifkova for her assistance in reviewing the Carolina Biological slide collection.

References


IX. Vision
Mapping of Rods and Cones

Materials
Large protractor
4 color cards (red, green, yellow, and blue)
Partner

Purpose
This experiment demonstrates two aspects of vision. First, rod and cone systems have varying degrees of sensitivity. This feature is easily demonstrated by comparing the angle at which an object is observed in the periphery with the angle at which you are able to recognize the object and discern its color. Second, this experiment demonstrates the distribution of the different types of cones across the retina by determining the angle at which different colors are first identified when they are brought into peripheral vision.

Background
The transduction of light into a neural message occurs when the stimulus enters the eye and strikes a layer of photoreceptor cells on the retina. Special structures inside these cells are filled with a pigment that changes in response to light. This pigment is called rhodopsin. Rhodopsin is made of two smaller molecules: retinal and opsin. (Retinal is related to vitamin A, which is why a deficiency of the vitamin can sometimes lead to night blindness). Retinal contains two molecular chains in a cis conformation (shape) around one of its double bonds (Figure 1). This means that they lie on the same side of the double bond. Ordinarily, these two bulky chains would “repel” each other (steric hindrance: the large atoms in these bulky chains destabilize the molecule when they are too close in space) and rotate to a more stable state across the bond (to a trans conformation). One characteristic of the double bond is that it resists rotation, so the unstimulated molecule keeps the less stable cis (same side) conformation.

FIGURE 1

1 Contributed by J. Hammock, Fall 1996
The energy created by light striking the retina briefly frees the retinal double bond and allows it to change to its more stable all-trans conformation. As a result of this shape change, retinal breaks apart from the opsin to which it was bound.

When retinal separates from opsin, an activated form of the larger pigment, rhodopsin is formed. Activated rhodopsin stimulates a G protein, which, in turn, activates an enzyme called cGMP phosphodiesterase (cGMP phosphodiesterase breaks down cGMP). Photoreceptors contain sodium channels on their plasma membranes that are held open by the presence of cGMP. In the absence of light, these channels are open allowing sodium to enter the cell (so that in the dark, the photoreceptor is in a constant state of depolarization). However, when light strikes the retina it generates the above mentioned cascade which decreases the concentration of cGMP. The decrease in cGMP created by light actually hyperpolarizes the cell by closing the sodium channels. This hyperpolarization is the neural signal interpreted as light (see Figure 2 for a summary).

![Illustration of retinal and photoreceptor processes](image.png)

**FIGURE 2**


The retina consists of two types of photoreceptor cells, rods and cones (Figure 3). Rods are the more sensitive photoreceptors, responding to a smaller intensity light stimuli of a broader spectrum. However, rods are achromatic (not sensitive to color). Cones require a greater intensity stimulus, and respond only to light of a particular wavelength. There are three types of cones. The first responds only to short wavelength light (the wavelengths discussed here are all relative to the visual spectrum: ≈400-700 nm). These cones are sometimes called S cones, or blue cones because these wavelengths correspond to the blues and violets of the visible spectrum. Second are M or green cones. These cones respond to medium wavelength light. Finally, there are L or red cones. These cones respond to long wavelength light.
The presence of these three types of cones accounts for color vision. Different combinations of these receptors respond to different stimuli. Information from these receptors travels down ganglion cell axons (which make up the optic nerve) to the lateral geniculate nucleus of the thalamus. Here the information is relayed to visual cortex where the different combinations of stimulated cells are interpreted as color.

Method

There is a differential distribution of cones and rods across the retina. Furthermore, there exists a difference in the distribution of the three different types of cones. The first part of this experiment will allow you to roughly plot the relative location of rods and cones on the retina. The second component of the experiment will allow you to create a rough one dimensional map of the location of the three cone types on your retina.

1) Put the large protractor on the table and place your chin on the “x” (see Figure 4). Place a pencil at the 90° point on the arch. You should focus on the pencil during all of the tests.
2) Have your partner shuffle the color cards and choose one randomly (keeping the color identity concealed).

3) Your partner should place the color card at $0^\circ$ and slowly move it towards $90^\circ$ along the arch of the protractor.

4) *Remember to continue to focus on the pencil.* When you are able to see the card (but not necessarily its color!), alert your partner, but remain focused on the pencil.

5) Have your partner write down the angle at which you recognized the “colorless” card.

6) Have your partner continue to move the color card along the arch of the protractor.

7) When you are absolutely positive of the *color*, call out the color’s name (sometimes colors appear differently in the far periphery so don’t guess!)

8) When you have correctly identified the card’s color, again have your partner stop and write down the angle.

9) Perform the experiment enough times to complete the chart below. (Note: you only need to perform the “colorless” object identification test a total of three times for each eye; it doesn’t matter what color card you use for this aspect of the text).
### Results Questions

10) Using a black colored pencil, draw a line representing the average point of first recognition for the “colorless” object on the axis below.

11) Using colored pencils, draw a similar line representing the average point of first recognition for each color.

<table>
<thead>
<tr>
<th>“Colorless”</th>
<th>Red</th>
<th>Green</th>
<th>Blue</th>
<th>Yellow</th>
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<tbody>
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<tr>
<td>AVG.</td>
<td>AVG.</td>
<td>AVG.</td>
<td>AVG.</td>
<td>AVG.</td>
</tr>
</tbody>
</table>

\[ \text{LEFT} \quad \text{RIGHT} \]

\[ \begin{array}{cccccccccc}
90 & 75 & 60 & 45 & 30 & 15 & 0 & 15 & 30 & 45 & 60 & 75 & 90 \\
\end{array} \]

**Results Questions**

1) What photoreceptor type was most sensitive to objects in the periphery (remember, cones are being used only when color information is recognized)?

2) Between the lines you drew for each color is your field of vision for that color (at least along one axis). That is the area where cones responding to that color are found.
   - A. What color has the smallest field of vision?
   - B. What color has the largest field of vision?

3) As a demonstration, start the color card with the smallest field of vision at the 90° point, and move it towards 0°. When the color disappears, stop and note the angle.
   - A. Was it a larger or smaller angle than you reported before?
   - B. Is this a larger or smaller field of vision for that color?
C. Why do you think the field of vision changed (if it did)?

**Study Questions**

1) How does light become a neural impulse (list the stages)?

2) Given your answer in #1, what is the ONLY light dependent stage in vision (i.e. what stage, if eliminated, would eliminate a neural impulse)?

3) What is the difference between rods and cones?

4) What kinds of cones are there?

5) Note that any neural signal originating from the eye will be interpreted as light. This can be demonstrated by closing your eye and (gently!) pressing on your eyelid. Occasionally the pressure will result in a flash of light.
   A. What is occurring?

   B. What type of “neural coding” is consistent with this phenomena?

6) Sometimes when you look directly at a very dim star that you can see in your peripheral vision, it disappears. Why?

7) What evolutionary pressures may have resulted in the particular wavelength sensitivities of rods and cones?

8) Why is a three-cone system used (i.e. why not one or two, why not four)?

**References**

X. Morris Water Maze
A Test of Behavior

Materials

Maze: Circular Washtub -4-5' in diameter with -2' high walls.
Divide into four quadrants with string or wire stretched across the top.
Place a height adjustable, circular platform (-8" diameter) in one quadrant.
Fill with water to about 1 cm. above the platform.
(Rats should stand comfortably on the platform)
2 stopwatches
2 volunteer "timers"
1 volunteer "recorder" with paper and pencil
4 rats
4 Cues (large designs drawn on paper and hung on the edge of the maze, visible to the animal) and drugs (scopolamine) are optional.

Background

The Morris Water Maze Task is a behavioral task, that is, the dependent measure is the animal's behavior. Behavioral tests measure the responses of whole animals, allowing experimental manipulations to affect in vivo systems operating in harmony to provide a clear understanding of the outcome. Because there are so many factors involved between experimental manipulations and behavior, the effects can sometimes be unpredictable, and it can be difficult to ascertain whether the behavior was indeed a main effect of the experiment. Still behavioral experiments offer great insight into the effects of experimental manipulations on whole organisms.

One challenge in the study of learning and memory is that it can never be directly observed and must therefore remain a theoretical construct. Because of this limitation one can only infer that learning has occurred through an enduring change in behavior in response to an environmental stimulus.

A task that has been developed to study learning in animals is the Morris water escape task (the Morris water maze). This task uses a round pool of water in which a platform is submerged beneath the surface. When placed in the maze the animal's task is to find the hidden
platform. The experimenter can chart the "learning" of the animal by the time it takes to find the platform over a number of trials.

The following graph is typical of a rat who has learned the location of the platform:

By observing a change in the animal's behavior when it is repeatedly placed in the water, the experimenter can conclude that learning has occurred.

There are two basic forms of the Morris water escape task:
1) In the cued version the platform is visible to the animal.
2) In the non-cued version the platform is not visible. The animal must learn the platform's spatial position in the surrounding environment in order to escape.

These two distinctions are critical to interpreting results obtained from the task. Suppose an experimenter wanted to study the effect of a drug on learning. The experimenter injects one group of animals with the drug and another group with a vehicle (vehicle is the solvent that the drug is dissolved in). If the experiment showed these results:
the experimenter might be tempted to conclude that the drug impaired the animals' ability to learn. However, there are many alternative explanations for this hypothetical data set. For example, what if the drug impaired the animals' ability to see cues around the room? Decreased vision could impair the animals' ability to learn the task, but this is not a direct effect of the drug on learning and memory per se. Another possibility is that the drug lessened the animals' anxiety in the water and therefore decreased the desire to escape. In this case the animal might know where the platform is, yet wouldn't go there. The difference in performance would not be a result of learning, but would now be attributable to motivational factors.

At this point the researcher might run the same groups (drug and vehicle) in a cued version of the Morris water escape task. If the two groups show the same latencies to swim to the visible platform, then the experimenter can confidently conclude that:

1) The drug is not impairing the animals' vision.
2) The drug is not changing the motivation to escape the water.

Once alternative explanations such as these have been ruled out, the experimenter is closer to concluding that the drug impaired learning.

'The Morris water escape task has been used extensively in the study of learning and memory. One learning theory that this task is useful for testing is Rudy and Sutherlands' theory regarding the role of the hippocampus in learning and memory (1989). Rudy and Sutherland suggest that different learning paradigms can fall into two main categories:

1) Those that only require elemental associations.
2) Those that require more complex configural associations.

Tasks that use elemental associations are ones in which a response made by an animal to a given cue will always produce the same outcome. For example, if a rat is trained that in the presence of a tone he will receive food for pressing a bar, but will not be rewarded for pressing the bar in the presence of a blinking light, he has made elemental associations. The meanings of the cues (the result of bar pressing during the tone and blinking light) in this example are unambiguous. In contrast, the meaning of the cues in a configural association is ambiguous. The animal must learn the meaning of some cues in relation to other cues in the environment.

Take the tone and blinking light from the elemental association example. The experimenter could add a new set of cues to these and create a learning paradigm that requires configural associations. The animal could be presented with either the tone or blinking light in a black or a white box. In the black box, the animal will be rewarded for responding in the presence of a tone and not rewarded for responding in the presence of the blinking light.
Conversely, in the white box the animal will be rewarded for responding to the blinking light and not rewarded for responding to the tone.

In this scenario the animal can be reinforced in the black and the white box, and it can be rewarded for responding to both the tone and the blinking light. It must, however, be sensitive to the relationship between the four different cues to be rewarded at all. When the cues in a learning paradigm have ambiguous meanings like this example, the animal must develop configural associations to link their meanings together.

<table>
<thead>
<tr>
<th>Black Box</th>
<th>White Box</th>
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<tbody>
<tr>
<td>Tone- Rewarded</td>
<td>Tone- Not Rewarded</td>
</tr>
<tr>
<td>Light- Not Rewarded</td>
<td>Light- Rewarded</td>
</tr>
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</table>

Most learning paradigms that require configural associations require a fully functioning hippocampus, however learning paradigms that can be solved using only elemental associations can be solved without input from this structure.

The Morris water escape task can test Rudy and Sutherland's theory on the role of the hippocampus in learning and memory. With the platform submerged underneath the surface of the water one can make animals solve the maze using either elemental or configural associations. This can be done by simply changing the animals' starting points. If the animal is started in the same position (or quadrant of the pool) at the beginning of each trial, then it will only need to use elemental associations to find the platform. This is because the animal can choose one cue in the room and the platform will always be in the same place in relation to that cue. If the animal is started in a different position for each trial (different random quadrants), it can no longer rely on finding the platform in relation to one cue. The animal now must build a cohesive spatial representation of the room to find the platform. The animal may swim towards the door on one trial, yet have to swim away from it the next trial. Because the platform no longer can be found using one visual cue, the animal must construct configural associations to solve the task.

Would a rat with hippocampal damage be able to solve the task when started from the same quadrant? different quadrants?
Method

1) Hand a stopwatch to each volunteer timer. One timer will be responsible for measuring the time it takes the animal to find the platform once it is placed in the water. The other volunteer measures the amount of time spent in the quadrant containing the platform.

2) Number the animals' tails. Place the first animal on the platform for about 20 seconds. This will let the animal observe the cues in the room and their relationship to the platform. After the 20 seconds, gently place the animal in the water in one of the quadrants with its head facing the wall. Timers will begin recording now. Make sure to record which quadrant the animal is placed in (you will want to place animals in all of the quadrants in random order). If on any trial the animal does not find the platform in 60 seconds, the first timer should notify the T.A. who will remove the animal from the water and place it on the platform.

3) Record the data onto the chart included with this lab.

4) Once the animal finds the platform, allow it to sit for 5 seconds and then remove it from the maze. Record the initial quadrant it was placed in, the time required to find the platform, and the time spent in the platform's quadrant.

5) Follow this procedure for the other three animals. You are now ready for trial 2. Repeat the procedure (you can omit placing the animal on the platform before placing it in the water, but continue to allow it 5 seconds after it has found the platform). Make sure you place the animal in a different quadrant for each trial. Continue running trials for all of the animals until they can all repeatedly find the platform in less than 5 seconds.

6) Once all of the animals have learned the task (they are all finding the platform in less than 5 seconds), there are a number of behavioral questions that can be answered. For instance:

   - What cues might the animal be using?
     If you place internal cues in the maze (hanging drawings), change their position. Does the animal take longer to find the platform? What quadrant is it spending most of its time in?
     Perhaps it is using the people in the room, try the test after everybody switches places.

   - What if you were to rotate the maze?
     Move the island to a new quadrant. Notice the amount of time spent by the animal in each quadrant. How long does it take for the animal to "relearn" the task?

   - The drug scopolamine often impairs performance on this task. If the drug is available (as well as a protocol), give some to the animals after they learned the task, what happens? What can you conclude from this data?

   - Spend some time thinking of your own experiments!
7) On a separate sheet of graph paper graph the learning curve for each animal. The x-axis should represent trial number while the y-axis should represent time to platform (TTP).

**Review Questions**

1) What are some advantages and disadvantages of behavioral experiments?
2) Describe original examples of elemental and configural associations.
3) What role does the hippocampus play in Rudy and Sutherland's theory? What else does the hippocampus do?
4) What other factors besides learning could cause poor performance in the Morris Task? How do you account for these factors?
TTP – Time to Platform
PQ – Time spent in platform's quadrant

<table>
<thead>
<tr>
<th>Trial</th>
<th>Quad</th>
<th>Animal 1</th>
<th>Animal 2</th>
<th>Animal 3</th>
<th>Animal 4</th>
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<tbody>
<tr>
<td></td>
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<td>TTP</td>
<td>PQ</td>
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