Fall 2018

Nsci 2100: Human Neuroanatomy Lab manual

CONTENTS:

Useful online resources:

Sheep brain:
https://www.msu.edu/~brains/brains/sheep/exterior/inferior.html

Human brain:
http://www.anatomie-amsterdam.nl/sub_sites/anatomie-zenuwwerking/123_neuro/start.htm

Rodent brain (sections):
http://mouse.brain-map.org/static/atlas

INTRODUCTION

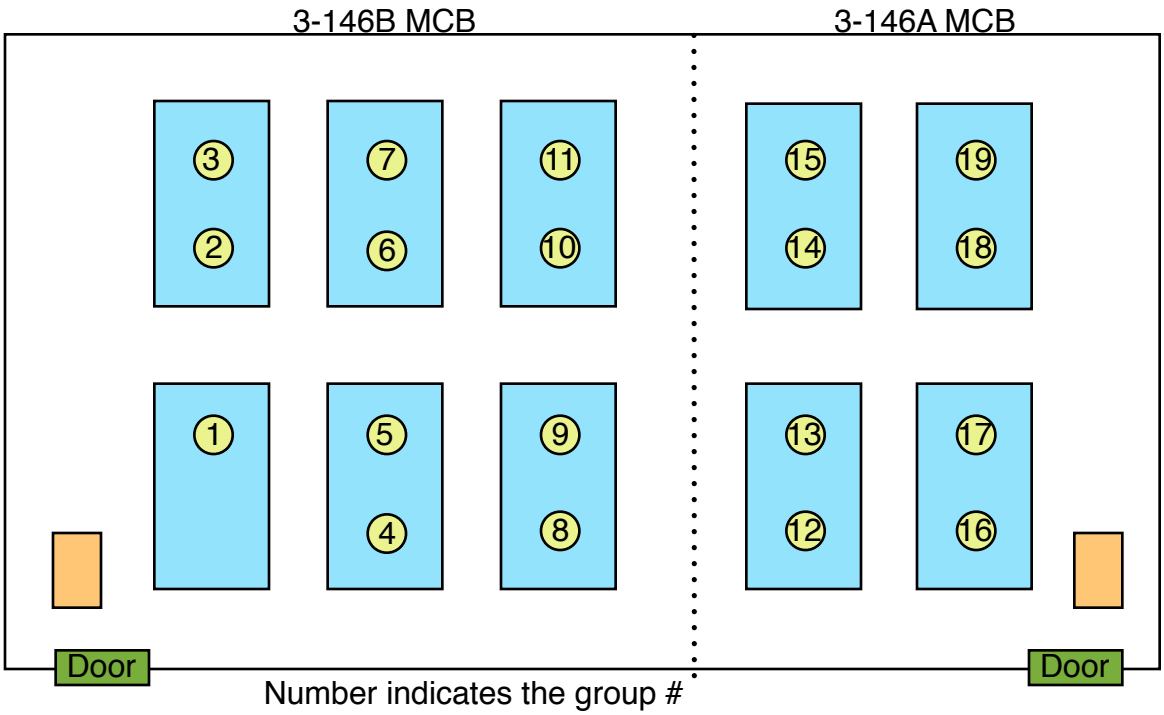
This lab is an essential part of the course and supplements the material covered by the lectures. You will examine the structure and functions of the nervous system by a variety of approaches.

Lab Director:
Dr. Maureen Riedl (riedl003@umn.edu)

Instructors:
Dr. Dezhi Liao (liaox020@umn.edu): Section 2 (Mon 10:10am), Section 3 (Mon 2:30pm)
Dr. Maureen Riedl (riedl003@umn.edu): Section 4 (Mon 2:30pm)
Dr. Wensheng Lin (linw@umn.edu): Section 5 (Tue 1:25pm), Section 6 (Wed 10:10am), Section 7 (Thu 10:10am)
Dr. Martin Wessendorf (wesse001@umn.edu) Section 8 (Thu 1:25pm)

Teaching Assistants:
Section 2: Victoria Rogness, Cailin Hildebrand
Section 3/4: Lauren Bystrom, Jonathon Kingsbury, Olivia Korenfeld
Section 5: Madison Tetzlaff, Kayla Switalla
Section 6: Miranda Severin, Kyle Schroeder
Section 7: Ryan Topp, Neema Afshar
Section 8: Israel Falade, Tyler Scharber
Lab coordinator: Kayla Switalla

Lab Location:
MCB 3-146A/B (Sections 3/4)
MCB 3-146B (Section 2,5,6,7,8)
LAB 11 (this is an optional lab and you are not required to attend or submit a lab report) will be held in the Anatomy Lab in Jackson Hall (Room number to be announced)



-Attend every lab section at the day and time you have registered for.

-The lab manual will be downloadable in advance from the course website under “Lab Manual”. Make sure you read the manual before coming to the lab.

-We will take attendance at the beginning of the lab. There will be an attendance sheet you need to sign in. If you do not sign in, you will not be given credit for that lab session.

-A short lab report is required for most labs. Its due date is shown at the beginning of each lab manual. For each late report, 2 points per week will be subtracted (if the report is turned in 2 weeks after the due date, you will get zero point for that lab).

-The point system for the lab section of this course is as follows:
attendance: 1.0/lab
lab report: 0.0-4.0/lab
total point for each week: 5.0

-In case you are unable to attend the lab for a valid reason (e.g., sickness), notify the faculty instructor of your section by email as soon as possible so that you get the attendance point for the lab. Even in this case, you are required to turn in the lab report. Arrange for a makeup session with your section instructor.

-The final lab “Human Cadaver” is optional, and there is no point associated with this lab.

-Each section has ~30 students. You will be split into groups of three students who will share the materials and supplies. Include the group number in lab reports. See previous page for the lab layout and group numbers.

-For some labs, we will loan each group an iPad. It is for use in the lab only. Do not make any modifications to the iPads.

-Be very careful not to damage the lab materials except when you are allowed to dissect sheep brains. Human brains and spinal cords are shared with medical student labs and are very difficult to obtain; treat them with a special care!

-Out of respect for the individuals and their families who donated their bodies at death for our use, no photography of human tissue is allowed in the laboratories.

Bring to each lab:
✦This lab manual (either in print or on your portable electronic device)

✦Notebook: *Student Lab Notebook – Life Sciences* with spiral binding and 70 carbonless duplicate pages (ISBN 978-1-930882-35-5)

✦The textbook (optional): *Essential Neuroscience,* by A. Siegel & H.N. Sapru (ISBN 978-1451189681). In this manual, we refer to figures in this book (e.g., “see S&S, Fig.1-2)

✦Pen or pencil

-We provide gloves as well as necessary supplies and tools.

-Do not bring food or drink in the lab (even water bottles). Wear close-toed shoes and full-length pants or skirts in the lab.

Lab report
-Due date for each lab report is specified in the manual.
-Turn in the original pages and keep the copy pages.
-No report is required for LAB 11 (Human Cadaver lab; attendance is optional).

-how to organize your report in the lab notebook:

LAB1	**Overview of the vertebrate nervous system**		9/14/18
Your name	Your lab partners	Group 2	Section 2

On this side of the page, answer the questions at the end of each lab manual.	Feel free to comment on the lab materials on this page. Describe what went well and what did not.

Your signature	9/21/18		

THE MAYDEN-McNEIL STUDENT LAB NOTEBOOK NOTE: INSERT DIVIDER UNDER COPY SHEET BEFORE WRITING

LAB 1: OVERVIEW OF THE VERTEBRATE NERVOUS SYSTEM

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET

Report of LAB 1 is due at the beginning of your LAB 3.

Objectives:
1. define the major coordinates of the brain and spinal cord as used in science and medicine
2. identify major brain regions in the intact brain, half brain and dissected brainstem
3. compare sheep and human brains

Materials:
1. whole **sheep** brains (without dura or blood vessels; one brain per group)
2. whole and half **human** brains (without dura and blood vessels)

NOTE: **Human** brain specimen are very precious and the same specimen are used in the Neuroscience Lab course for first-year medical students. Handle them with great care under the supervision of your instructor. You are also responsible for keeping the brains moist. The reason we use **sheep** brains in this course is that you can dissect and manipulate them as much as you want (according to our instructions) to better understand the three dimensional organization of the mammalian brain.

Brains are placed in formaldehyde solution for long-term storage. Just before use, sheep brains are rinsed in water to reduce the smell. You should still note some smell of formaldehyde as you handle the brain.

Your dissection tools:
brain knife (one per group: be careful. It is very sharp!)
scissors (one per group)
forceps (one per group)
magnifying glass (one per group)
other supplies: cutting board, blue pad, and gloves

Identifications (you will be asked to identify them on exams):
lobes of the cerebral cortex (frontal, parietal, temporal, occipital, limbic)
interhemispheric fissure (= medial longitudinal fissure)
hypothalamus
thalamus (gateway of sensory and motor information to the cerebral cortex)
pineal body (produces melatonin; controls the circadian rhythm)
brainstem
- midbrain (including superior colliculus and inferior colliculus)
- pons, medulla

cerebellum
spinal cord
cranial nerves and their tracts (there are a total of 12 pairs of nerves)
- olfactory tract (conveys information on smell to the brain)
- optic nerve, optic chiasm, optic tract (from the retina to the brain)
- oculomotor nerve (controls eye movement and light reflex)
- trigeminal nerve (sensations in the face)
- abducens nerve (moves the eyes outwards (abduction))

major axon tracts:
corpus callosum (connects right and left hemispheres of the cerebral cortex)
cerebral peduncles (from the cerebral cortex to the brainstem and spinal cord)

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

See p.13 for details.

Procedure:

1. Place a cutting board on a blue pad on your lab bench. Wear a pair of gloves and put a whole sheep brain on the cutting board. Observe the whole sheep brain. Determine the axes.

Axes of the brain
anterior (rostral)-posterior (caudal)
dorsal-ventral

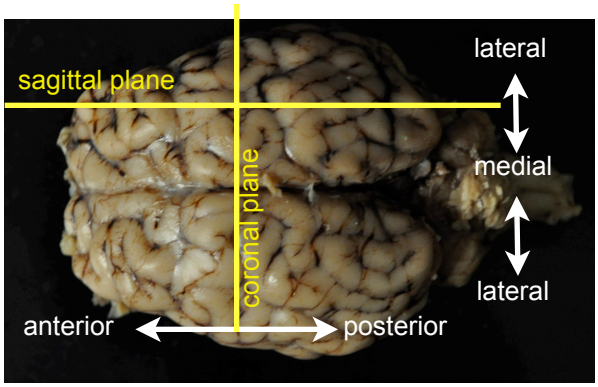


Fig. 1-1. dorsal view of a sheep brain

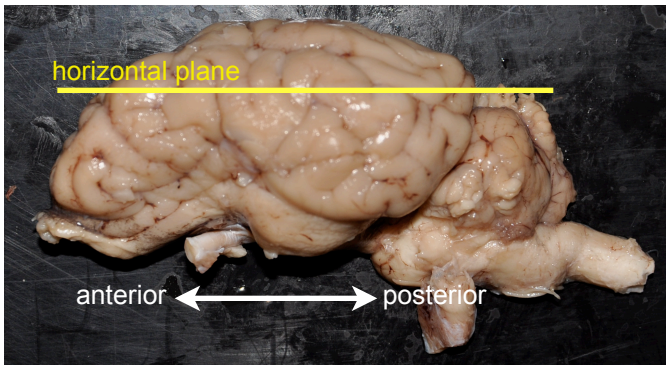


Fig. 1-2. lateral view of a sheep brain

These terms are not only used for structures (e.g., medial hypothalamus, caudal midbrain); they are also used for various views of an anatomical structure (e.g., dorsal view, medial view, etc.).

Brains can be sectioned in different orientations:
Examples:
coronal sections
sagittal sections
horizontal sections

2. Observe the whole sheep brain in three views (dorsal, lateral, ventral) and identify the structures listed.

Dorsal view

1. cerebral cortex
2. interhemispheric fissure (medial longitudinal fissure)
3. cerebellum (vermis)
4. cerebellum (hemisphere)
5. medulla

You will see that the cerebral cortex is divided into right and left hemispheres by the interhemispheric fissure (=medial longitudinal fissure: fissure means a large groove). The cerebral cortex is folded into ridges, called gyri (singular, gyrus) and furrows, called sulci (singular, sulcus).
The cerebellum consists of medially placed vermis (Latin for worm) and the laterally placed hemisphere. You should also find the medulla, which is located caudally to the pons and the cerebellum.

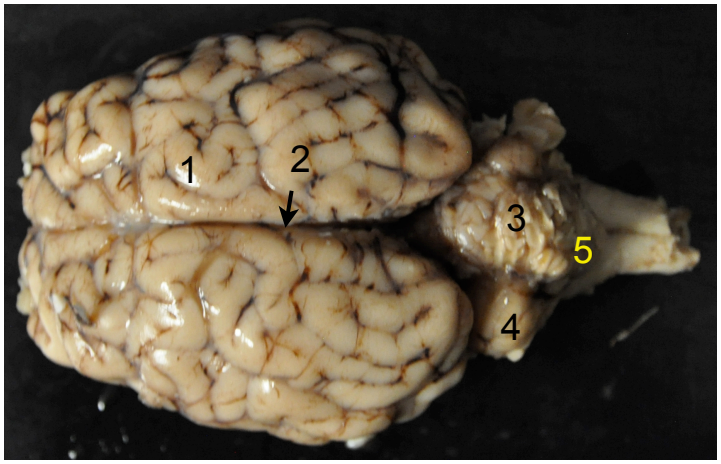


Fig. 1-3. dorsal view of a sheep brain

Lateral view

Lobes of the cerebral cortex	Other structures:
1. frontal lobe	5. cerebellum (vermis)
2. temporal lobe	6. cerebellum (hemisphere)
3. parietal lobe	7. pons
4. occipital lobe	8. medulla
(limbic lobe: not visible)	9. olfactory tract
	10. optic nerve
	11. trigeminal nerve

On lateral view (side view), find the four lobes of the cerebral cortex (frontal lobe, parietal lobe, temporal lobe, occipital lobe). Caudal to the cerebral cortex, you should see the cerebellum (vermis in the middle and hemispheres on the sides) and the brainstem, which includes the pons and medulla. You will also notice some cranial nerves, including the optic nerve and trigeminal nerve. The olfactory tract is a bundle of axons that is attached to the frontal and temporal lobes of the cerebral cortex.

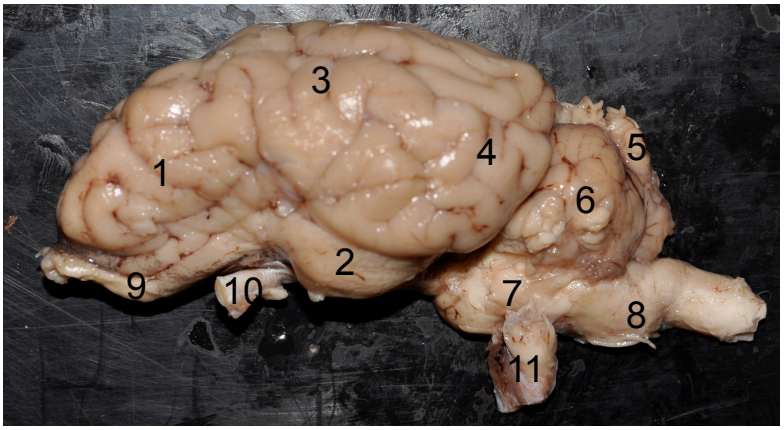


Fig. 1-4. lateral view of a sheep brain

Ventral view

1. cerebral cortex (temporal lobe)
2. optic nerve (2nd cranial nerve)

hypothalamus (ventral surface surrounded by dots)
3. optic chiasm
4. optic tract

midbrain
5. cerebral peduncle
6. oculomotor nerve (3rd cranial nerve)

hindbrain
7. pons
8. medulla
9. trigeminal nerve (5th cranial nerve)
10. abducens nerve (6th cranial nerve)

Ventral view reveals the underside of the temporal lobe of the cerebral cortex. In this view, the anterior-posterior division of the brain is easily seen: you should find the hypothalamus, the brainstem (midbrain, hindbrain (pons and medulla)) and possibly part of the spinal cord. Near the hypothalamus, you will find the optic nerves crossing the midline at the optic chiasm and continuing further caudally as optic tracts. The floor of the midbrain consists of a pair of cerebral peduncles separated by a space. You will find a large pair of nerves (oculomotor nerves) coming out of this space. Further caudally, you will find the ventral part of the pons and medulla, with a number of cranial nerves exiting and entering. Mammals have a total of 12 pairs of cranial nerves.

Warning: due to the handling during brain extraction by commercial suppliers, many of the cranial nerves may have been damaged already.

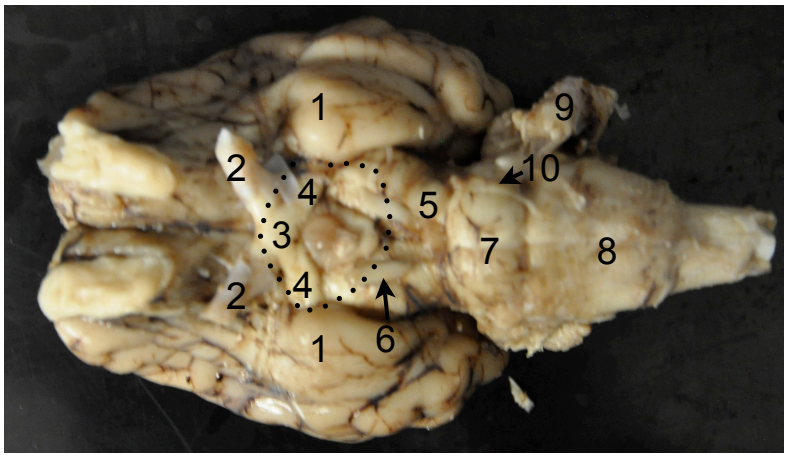


Fig. 1-5. ventral view of a sheep brain

3. Place the whole sheep brain on the cutting board (ventral surface down). Using a brain knife, carefully cut the whole sheep brain at the midline into right and left halves. Then the medial surface of the brain will be exposed. Now from the medial view, identify the listed structures:

Medial view

1. medial surface of the cerebral hemisphere (including the limbic lobe)
2. corpus callosum
3. thalamus
4. hypothalamus
5. pineal body
6. midbrain including the superior colliculus (sc) and inferior colliculus (ic)
7. pons
8. medulla
9. cerebellum (vermis)
10. spinal cord

Make sure that you keep the half brains in the plastic container clearly labeled with your group number. You will use these half brains next week (Lab 2).

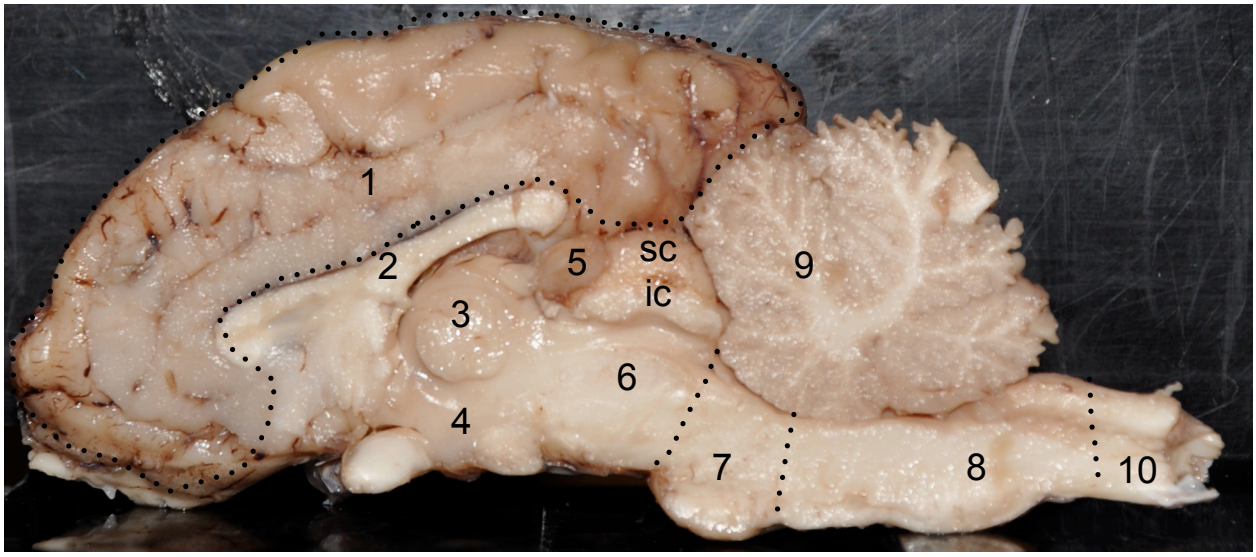


Fig. 1-6. medial view of a sheep brain

4. Compare the whole and half human brains with the sheep brain.

Observe the human brain and identify the structures that you found in the sheep brain. Compare the relative size of each structure and find any significant difference between sheep and human brains. For example, do you find any regions that are proportionally larger in the sheep brain than in the human brain? Why do think that is the case? Can you see the same gyri and sulci in the cerebral cortex of the human and sheep brains?

Lateral view

cerebral cortex
1. frontal lobe
2. temporal lobe
3. parietal lobe
4. occipital lobe
5. lateral sulcus
6. central sulcus

7.cerebellum (hemisphere)

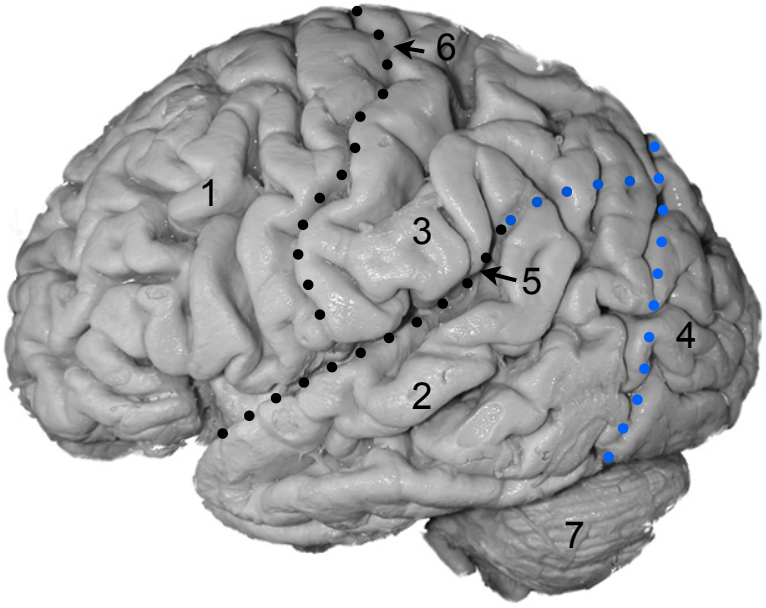


Fig. 1-7. lateral view of a human brain (see S&S Fig.1-2)

Ventral view

1.cerebral cortex (temporal lobe)
2.olfactory tract
3.optic nerve (cranial nerve II)
4.optic chiasm
5.cerebral peduncle
6.pons
7.oculomotor nerve (cranial nerve III)
8.trigeminal nerve (cranial nerve V)
9.abducens nerve (cranial nerve VI)

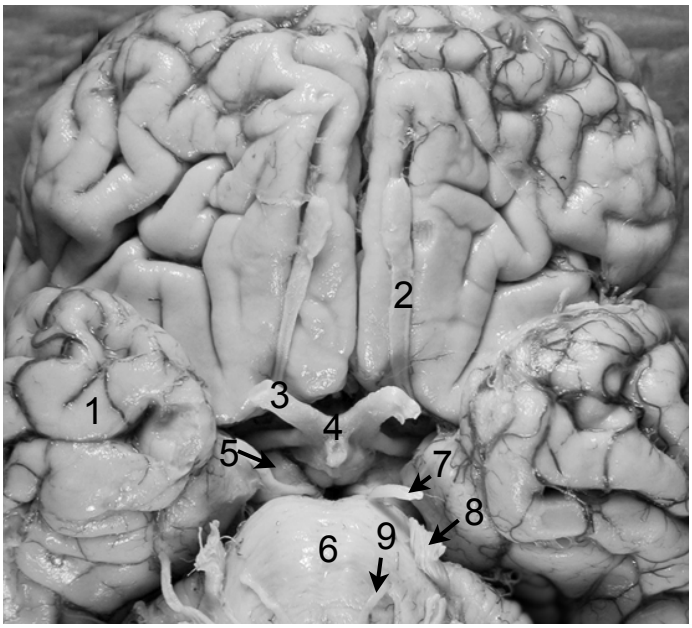


Fig. 1-8. ventral view of a human brain

Medial view

1. medial surface of the cerebral hemisphere (including the limbic lobe)
2. corpus callosum
3. thalamus
4. hypothalamus
5. pineal body
6. midbrain including the superior colliculus (sc) and inferior colliculus (ic)
7. pons
8. medulla
9. cerebellum (vermis)

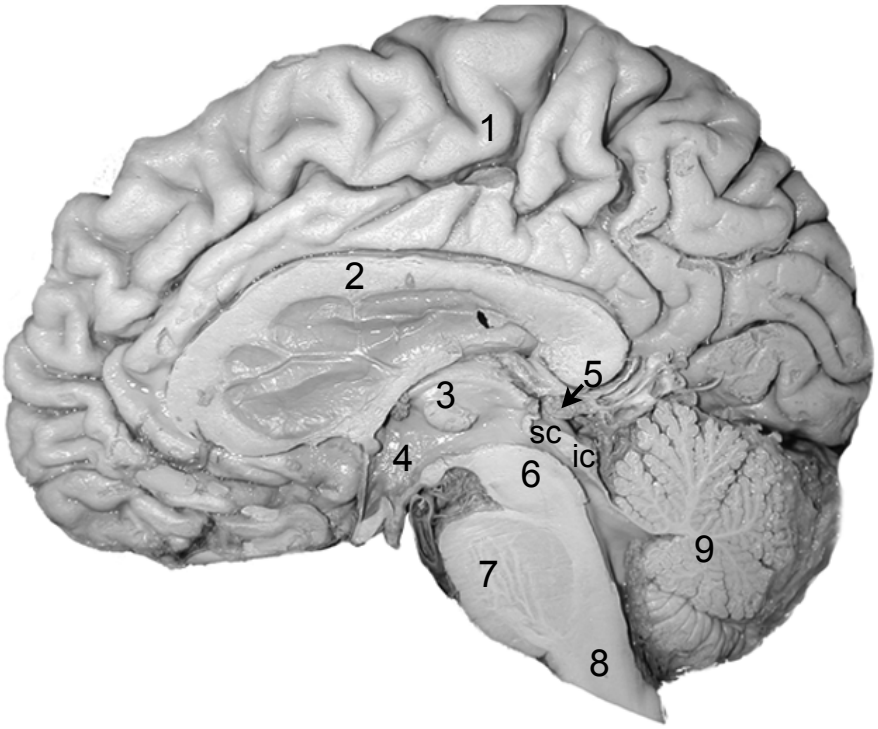


Fig. 1-9. medial surface of a human brain

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*

1. Draw the ventral and medial views of the sheep brain and the label the structures on the list (even numbered ones only) (2.0 points possible: 1.0 point for each view). 0.2 point for each structure. Indicate if you think certain structures are missing in your sheep brain.

2. Classify each of the following structures into one of the five subdivisions of the brain (telencephalon, diencephalon, brainstem, cerebellum and spinal cord). (2.0 points possible: 0.4 point for each item)

    occipital lobe
    thalamus
    pineal body
    superior colliculus
    pons

LAB 2: MENINGES, VENTRICULAR SYSTEM AND BLOOD VESSELS

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET

Report of LAB 2 is due at the beginning of your LAB 3.

Objectives:
1. understand the covering of the brain by the meninges (dura mater, arachnoid, pia mater)
2. identify ventricles and understand the flow of cerebrospinal fluid
3. identify the major arteries that supply blood to the brain and spinal cord

Materials:
1. whole sheep brains (with dura on; one brain per group)...you will dissect this brain by first removing the dura
2. half sheep brains (you prepared them in Lab 1; one pair per group)
3. whole human brains (with vasculature)
4. half human brains (3-4 per room)
5. dissected human brainstem (3-4 per room)
6. human spinal cord (3-4 per room)
7. plastic model of ventricles of the human brain (one per group; **be very careful in handling the model. It is very fragile**)
8. sheep brains injected with a dye in the vasculature (one brain per group; **do not discard these brains after you use them; they will be shared by different sections throughout the week**)

Your dissection tools:
scissors (one per student)
forceps (one per student)
magnifying glass (one per group)
scalpel (one per group: be careful. It is very sharp!)

Identifications (you will be asked to identify them on exams):
dura mater
arachnoid
lateral ventricle
third ventricle
cerebral aqueduct
fourth ventricle
foramen of Luschka
cerebellar peduncles
vertebral artery
basilar artery
circle of Willis
posterior cerebral artery
middle cerebral artery
anterior cerebral artery
anterior spinal artery

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

See p.21 for details.

Procedure:

1. [USE THE BRAIN WITH DURA] **Identify and remove the dura mater (the outermost layer of the meninges) from an intact sheep brain. Your instructor will show you a video that explains the procedure.**

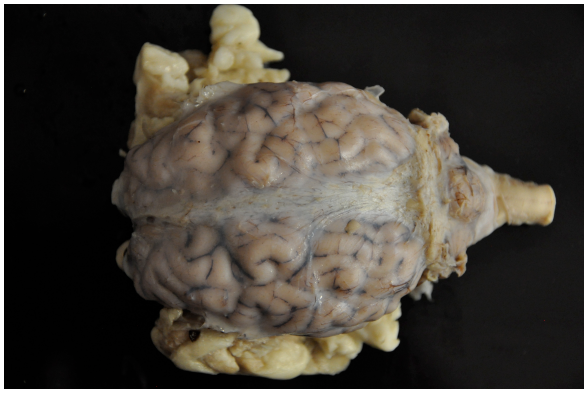


Fig. 2-1. dorsal view of the intact sheep brain (rostral is to the left)

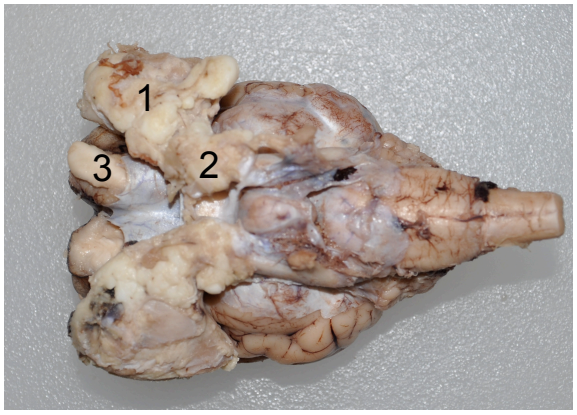


Fig. 2-2. ventral view of the intact sheep brain (rostral is to the left)

-First, place the sheep brain with the intact dura on the cutting board (Fig. 2-1).
Identify the thick covering of the brain (dura mater). Dura mater extends caudally and covers the cerebellum and brainstem. There are two prominent extensions of the dura mater: along the midline between the cerebral hemispheres and between the cerebral cortex and cerebellum. They serve to partition the space within the skull, further protecting the brain.

-Flip the brain upside down and put it ventral side up (Fig. 2-2). The dura covers the ventral surface of the brain as well. There is a pair of tissues with fat and muscle attached (1). This tissue is the remnant of the eye, and the optic nerve (2) comes out of it on each side. For now, leave this tissue intact

-Cut the dura between the right and left olfactory tracts (3). Next, for one side, cut the dura starting at a point lateral to the olfactory tract, advancing caudally along the lateral side of the cerebral cortex until you reach the posterior end. Repeat the cutting on the other side. Then lift the cut piece of the dura and remove it from the dorsal side of the brain. Flip the brain again. It will now look like the image in Fig.2-3.

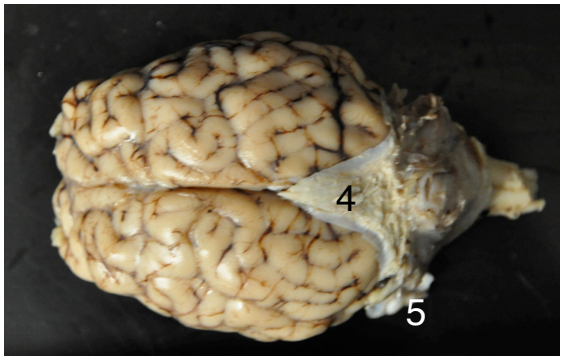


Fig. 2-3. dorsal view of the sheep brain after the dura is partially removed

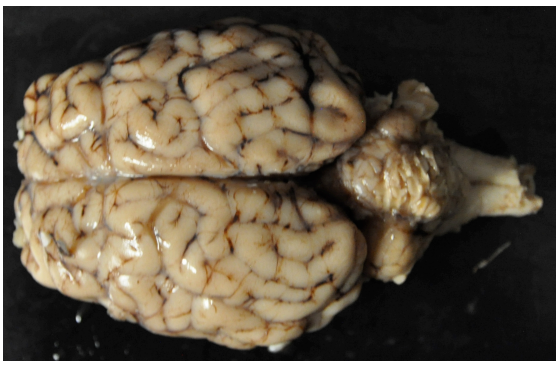


Fig. 2-4. dorsal view of the sheep brain after the dura is removed

-Find the hard, triangular extension of the dura (4) between the cerebral cortex and the cerebellum. Insert scissors into its lateral part (5) and advance medially to make deep cuts, making sure not to damage the brain itself (Fig. 2-4).

You should see a shiny membranous covering of the brain. This is the arachnoid. Major blood vessels are seen underneath the arachnoid (within the subarachnoid space). The inner-most layer of the meninges is the pia mater, but it is fused with the brain surface and cannot be distinguished from brain tissue with the naked eye.

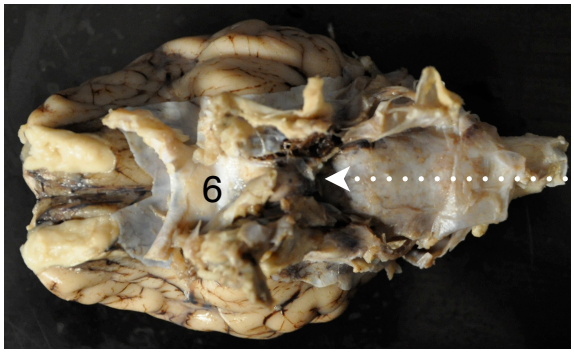


Fig. 2-5. ventral view of the sheep brain

-Flip the brain and put it ventral side up (Fig.2-5). Insert scissors into the dura from the caudal side of the medulla and cut the dura towards the rostral side (arrow in Fig.2-5). Be careful not to damage cranial nerves. A number of cranial nerves are attached to the ventral side of the brainstem (e.g., trigeminal nerve in the pons, abducens and facial nerves at the pons/medulla border). These nerves penetrate the dura, connecting the brain to the rest of the body; thus, lifting the dura very far will rip these nerves out of the brain. You do not want to cut these nerves too close to the point of attachment to the brainstem. As you encounter these nerves, cut them as close to the dura mater as possible so that you preserve the maximum length of the nerves.

-Remove the saddle-like thickening on the ventral midline that is immediately caudal to the optic chiasm and optic nerve (6). You can do this by inserting the scissors just medial to the trigeminal nerve (the nerve itself is tightly attached to this thickening).

-Advance the scissors rostrally and cut the thick tissue on both sides. You can now cautiously lift the cut tissue and find the oculomotor nerve exiting the ventral midbrain, as well as the infundibulum (the infundibulum attaches the hypothalamus and pituitary gland). To avoid ripping them out from the brain, cut he oculomotor nerves near the dura mater. Now remove the remainder of the thickening and expose most of the ventral surface of the brain. Cut the remaining dura near the olfactory tract.

2. [USE THE HALF SHEEP BRAINS YOU PREPARED IN LAB 1] **Place the half brains with the medial side up. Also look at a half human brain and a dissected human brainstem. Identify the major ventricles in these specimen.**

Ventricles to identify:
1. lateral ventricle (not readily visible in sheep brain; you need to remove the corpus callosum)
2. third ventricle
3. cerebral aqueduct
4. fourth ventricle

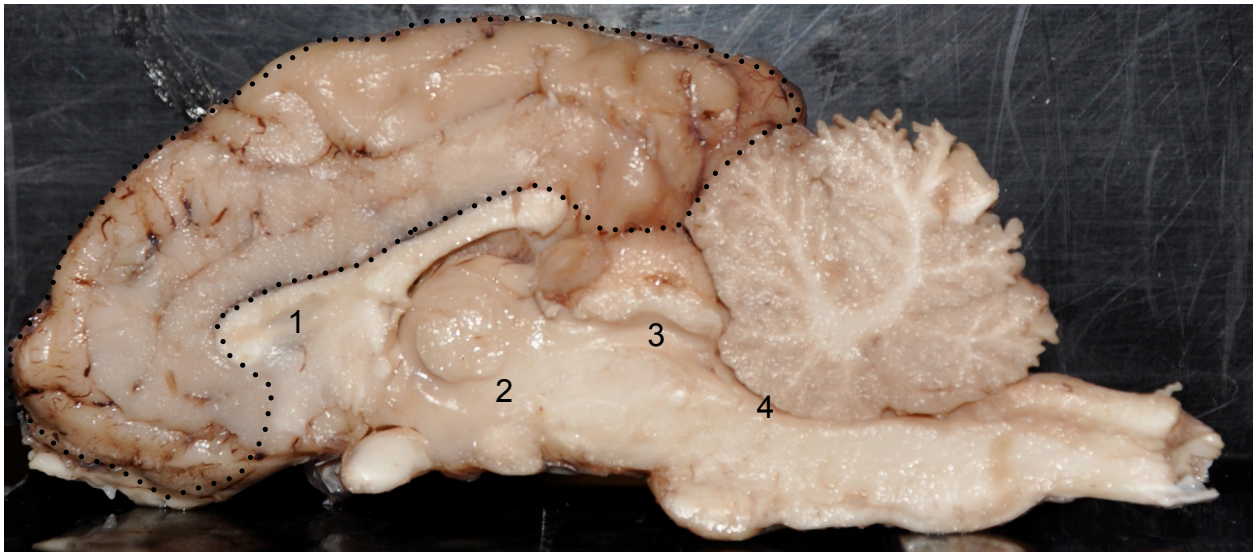


Fig. 2-6. half sheep brain

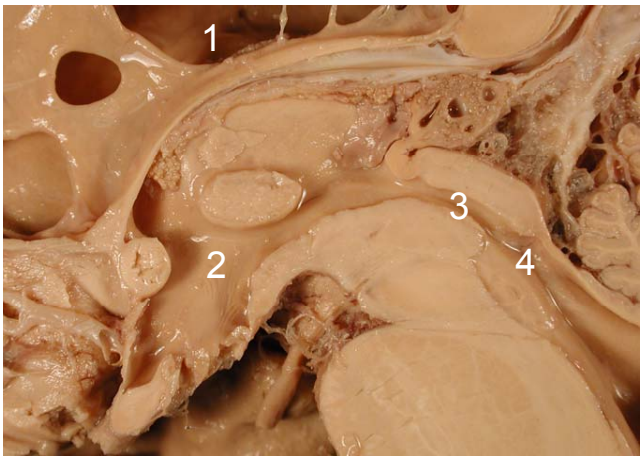


Fig. 2-7. half human brain

3. Identify structures near the fourth ventricle of the dissected sheep and human brainstem

Further dissect the whole sheep brain (the one from which you just removed the dura) to observe the brain ventricles. By holding the brain in your hands, identify the structures that connect the cerebellum with the brainstem (they are called the cerebellar peduncles). Cut them horizontally on both sides (parallel to the surface of the brainstem) using a scalpel (about 2cm deep). Then you should be able to lift the cerebellum off the rest of the brain. You will now find something similar to the dissected human brainstem.

Identification (on human brainstem):
1. fourth ventricle
2. foramen of Luschka (a pair of outlets through which the cerebrospinal fluid (CSF) leaves the ventricle and flows into the subarachnoid space: see Step 4 below)
3. cerebellar peduncles (superior, middle and inferior)

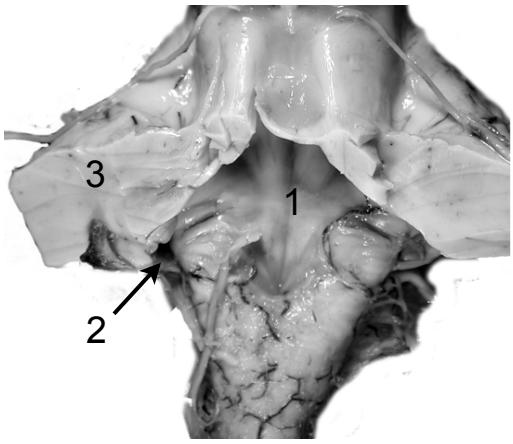


Fig.2-8. dissected human brainstem (dorsal view)

4. Use the ventricular model to understand how the ventricles are connected

The ventricular system is filled with cerebrospinal fluid (CSF). CSF is generated by the choroid plexus and flows from lateral ventricle, through the third ventricle, cerebral aqueduct and fourth ventricle. The CSF then exits the ventricle through three foramina (a pair of lateral foramina are called the foramen of Luschka, and there is also a single, medial foramen) into the subarachnoid space of the spinal cord and the lateral surface of the brain. Then it flows into the venous blood through the venous sinuses.

Review the CSF flow by observing the ventricular model.

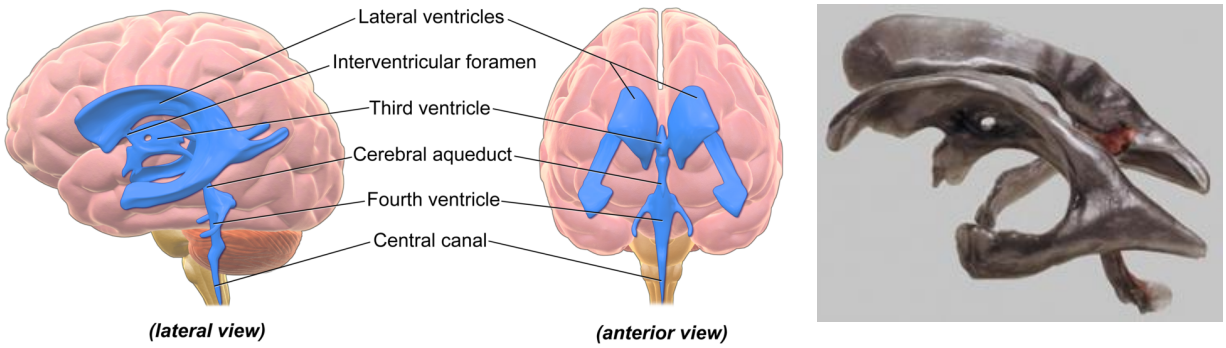


Fig.2-9. Overview of the ventricles and their model

4. Look at a whole and half human brain with vasculature and identify the major arteries.

Vasculature (use human brains)
internal carotid artery
middle cerebral artery
anterior cerebral artery
vertebral artery
basilar artery
posterior cerebral artery
circle of Willis

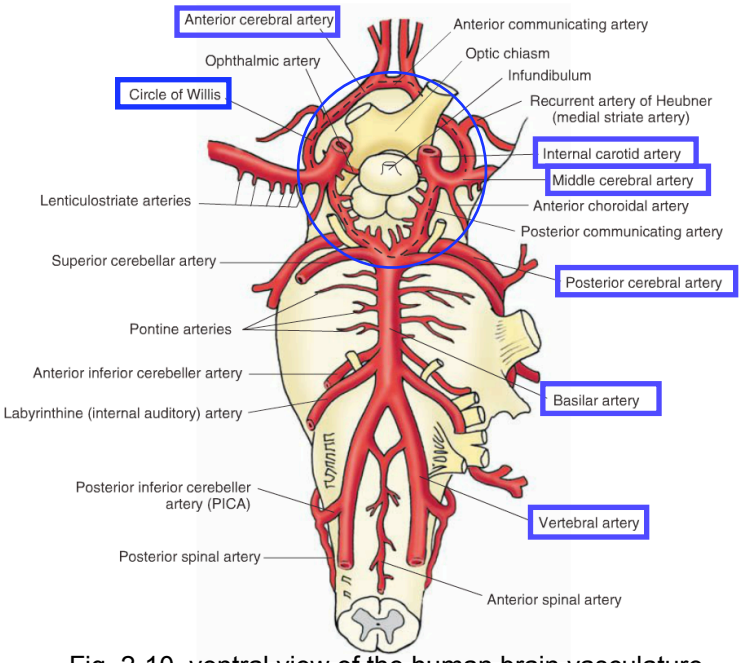


Fig. 2-10. ventral view of the human brain vasculature (S&S Fig.4-1)

circle of Willis (shown by a blue circle in Fig.2-10) consists of:

-posterior cerebral artery
-posterior communicating artery
-middle cerebral artery
-anterior cerebral artery
-anterior communicating artery

Can you identify similar arteries in the sheep brain? Use the sheep brain that has been injected with a red dye to identify the major arteries listed above.

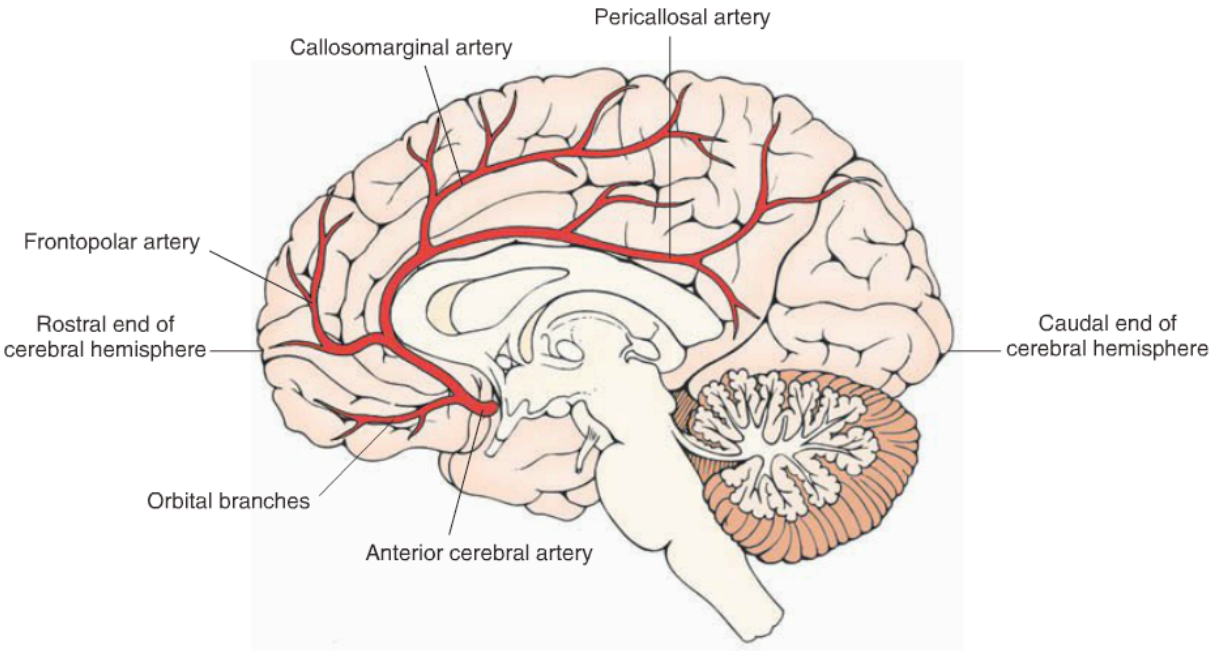


Fig. 2-11.medial view of the human brain showing branches of anterior cerebral artery (S&S Fig.4-2)

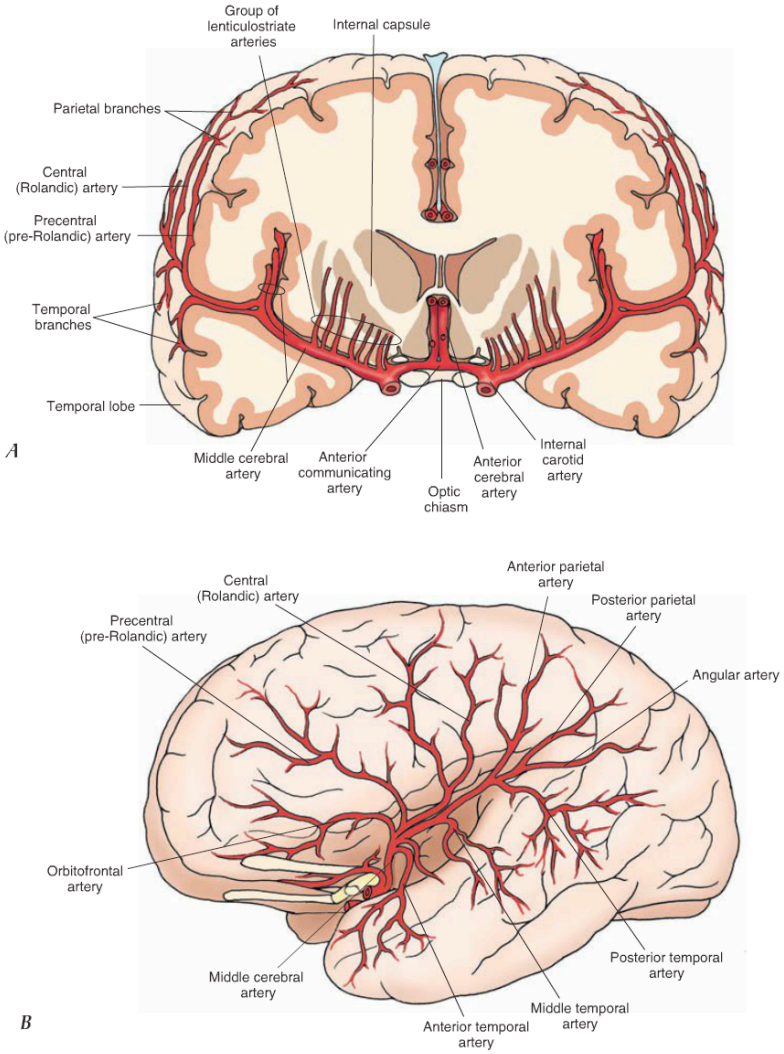


Fig. 2-12.lateral view of the human brain showing branches of middle cerebral artery (S&S Fig.4-3, p.50)

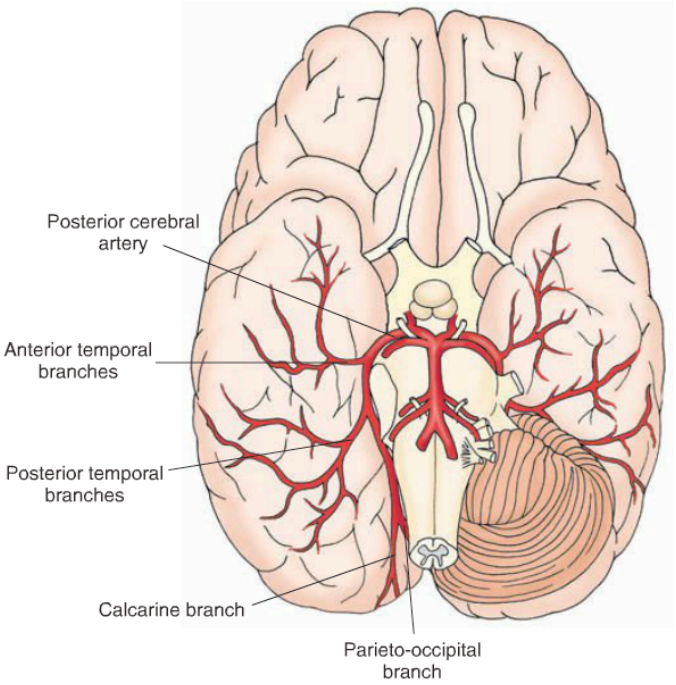
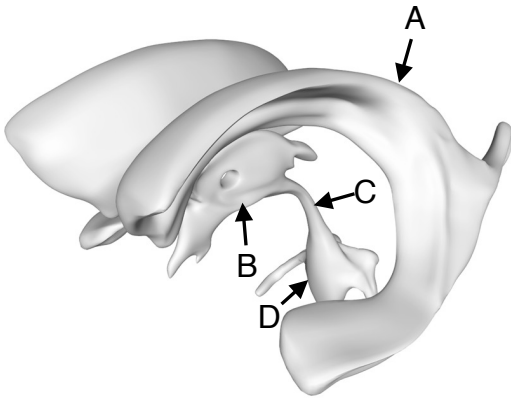


Fig. 2-13.ventral view of the human brain showing branches of posterior cerebral artery (S&S Fig.4-4, p.52)

<u>Lab Report (5.0 points possible for this lab)</u> *attendance point: 1.0, lab report point: 4.0*



1. Name each of the ventricles (A-D) shown in the schematic view on the right. (1.6 point possible: 0.4 point each)

2. For each of the ventricle you named in question 1, choose the structure from the list below that surrounds it. (1.6 point possible: 0.4 point each)

    spinal cord
    telencephalon
    midbrain
    medulla and pons
    diencephalon

3. Draw the dorsal view of the sheep brain after removing the cerebellum. Name structures that you can identify. (0.8 point possible)

    include the following:
        foramen of Luschka
        4th ventricle
        cerebellar peduncles

LAB 3: CELLS OF THE NERVOUS SYSTEM

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
TURN IN YOUR REPORTS FOR LAB 1 AND LAB 2

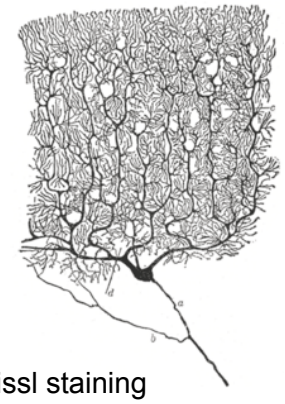
Report of LAB 3 is due at the beginning of your LAB 4.

Objectives:
1. Understand common features of cells in the nervous system
2. Understand the morphological diversity of neurons
3. Understand that neurons of the same type are grouped together in nuclei or layers

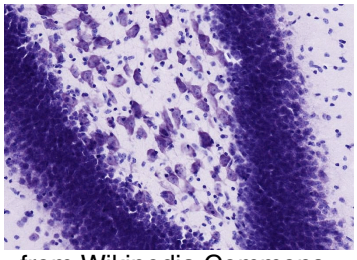
Materials:
1. Slides with mouse brain sections that were stained with two different methods:
   a. Golgi staining
   b. Nissl staining
   Slides for each staining method are provided in a plastic container (one container per group)

Your dissection tools:
microscope (with four objective lenses: 4x, 10x, 40x, 100x) one per group
iPad (one per group: for Allen Brain Atlas)

Golgi staining



Nissl staining



from Wikipedia Commons

Follow the procedure below and answer the questions in your lab report.

The Golgi method was developed by an Italian physician, pathologist and scientist, Camillo Golgi (1843-1926). After a special chemical treatment, blocks of brain tissues are immersed in a solution that contains silver nitrate. Grains of metallic silver crystalize inside some cells, producing a dense black precipitate that highlights the detailed structure of dendrites, cell bodies and axons. For unknown reasons, this method stains a small, random fraction of the billions of cells in the brain, which allows observation of the detailed morphology of single neurons. Santiago Ramón y Cajal (1852-1934) and others improved this method and used it to analyze the connectivity of the nervous system, which formed the basis of modern neuroscience.

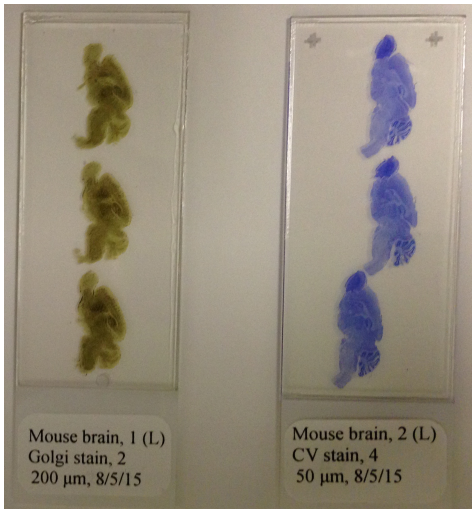
The Nissl method was developed by a German scientist Franz Nissl (1860-1919). It is a routine way of staining brain tissues. It can use a variety of dyes. The sections you will observe were stained by cresyl violet. This method stains charged structures (Nissl bodies) in the cell body violet. You can see that some parts of the brain have a higher density of stained cells than other parts. Such differences will help you identify various regions of the brain. We will use slides with Nissl-stained sections for orientation of the Golgi-stained slides.

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

See p.25 for details.

Procedure:

You will examine Golgi- and Nissl-stained sections of the mouse brain. Your lab instructor will briefly explain how to use the microscope. Each group will have have two small plastic containers. One contains slides of Nissl-stained sections. The other slide mailer contains Golgi-stained sections. Each slide contains three consecutive sagittal sections of adult mouse brains.



Golgi-stained brain #1(Left) slide #2 200μm-thick

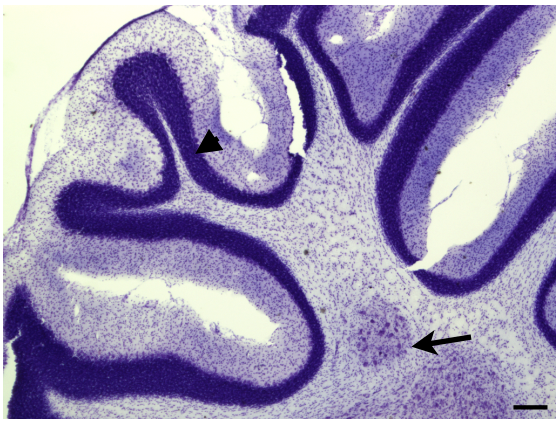
Nissl-stained brain #2(Left) slide #4 50μm-thick

Many of the sections on the slide will look like the diagrams in page 27-29. They show sagittal sections of the mouse brain at different medial-lateral levels (from Allen Brain Atlas (http://mouse.brain-map.org/experiment/thumbnails/100042147?image_type=atlas). Note that the structures shown are shared by all mammalian species, and most of them are even shared by non-mammalian vertebrate species.

In this lab session, you do not need to know the details of different parts of the brain (you will learn them in later labs), but it will be useful to become familiar with basic cell types in different brain regions as shown in the next pages.

1. Using the reference atlas in page 27-29 and **Nissl-stained sections** (labeled as "CV Stain": CV=cresyl violet), get a general orientation of the brain section you are observing. Use the iPad to look at a more complete of reference atlas at Allen Brain Atlas website (http://mouse.brain-map.org/experiment/thumbnails/100042147?image_type=atla). Start with the 4x objective to get a wide field of view. Slide #1 contains the most medial sections and slides with larger numbers contain more lateral slides.

2. Find the four regions shown below and identify the groupings of cells. Use higher magnifications objectives (10x or 40x; do not use x100 because it requires oil on the slide) to see the details of the cell packing.

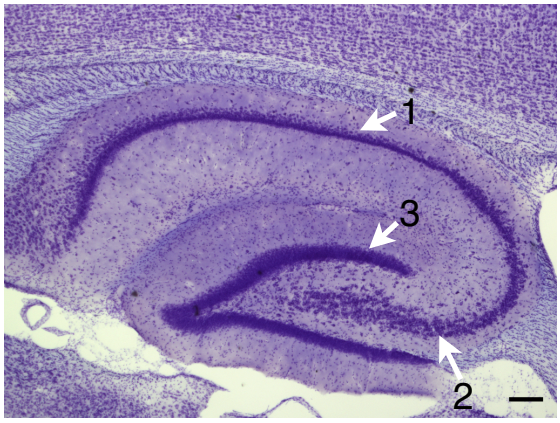


A. Cerebellum

Find the layer that has a very dark purple color in the cerebellum. This layer (shown by an arrowhead) has a high density of neurons called granule cells. This layer is called the granule cell layer. Find cluster of neurons in deep regions of the cerebellum. They are called deep cerebellar nuclei (shown by an arrow). The scale bar is 200µm long.

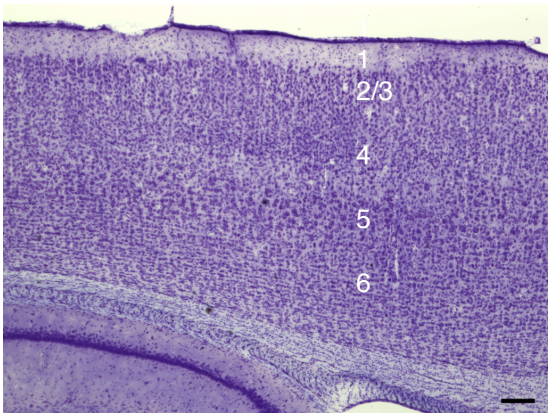
B. Hippocampus

Find layers of dark color in the hippocampus. They again contain neurons at a high density. Find major subdivisions of the hippocampus. As you see in the reference atlas, these are:
1: CA1 region
2: CA3 region
3: dentate gyrus



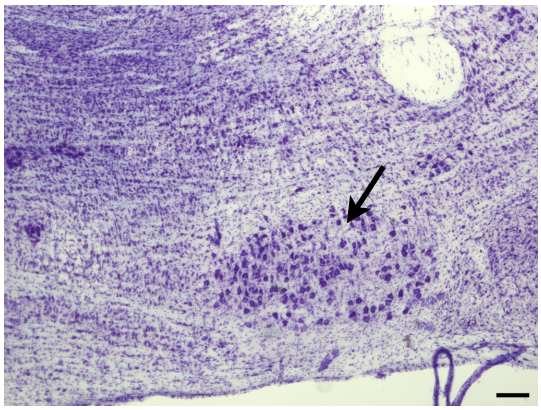
C. Neocortex

The neocortex has six layers of neurons. The most superficial layer is layer 1 and has a sparse population of neurons. Packing density and cell size differ between layers. For example, some layer 5 neurons have a very large cell body.



D. Medulla

Find a cluster of neurons with large cell bodies in the medulla (shown by an arrow). They are a group of motor neurons whose axons run in cranial nerves to control muscles.



3. Next, look at **Golgi-stained sections and sketch neurons**. This will be your lab report (see instructions below)

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*

1. From the four brain regions listed above, pick two of them. First, use the 4x objective and draw an image of each of these two regions. Show the name of this region, cell layers and nuclei, etc. (1 point possible: 0.5 point for each brain region)

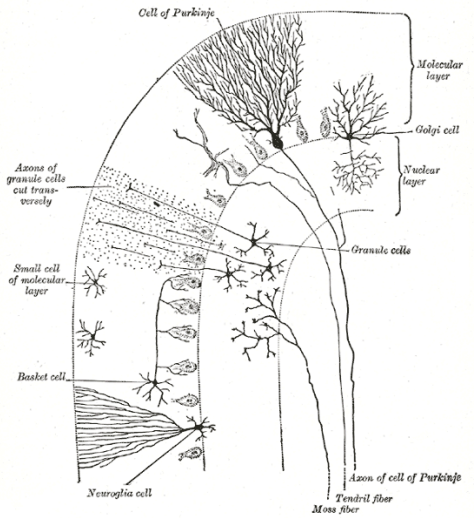
2. For each of the two brain regions you selected in question 1, use a high magnification objective (40x) and sketch **two** neurons that you think have distinct patterns of dendritic morphology. Show the location of each cell you draw in your low-magnification drawing (3 points possible: 0.75 point for each of the four cells to draw)

   Move the stage up and down to get a depth of focus so that you can draw the three-dimensional view of each cell onto a two-dimensional plane (your lab notebook). Describe in your words the morphology of the sketched cells. Some neurons may have a large number of spines on their dendrites (see the image below). Sketch those spines, too, if you can see them. For such neurons, dendritic spines are important for receiving synaptic input from other neurons. In lectures later in the course, you will hear about the roles of some of the neuronal types that you sketch in this lab.

dendrites with many fine spines (pyramidal neuron in the cerebral cortex)

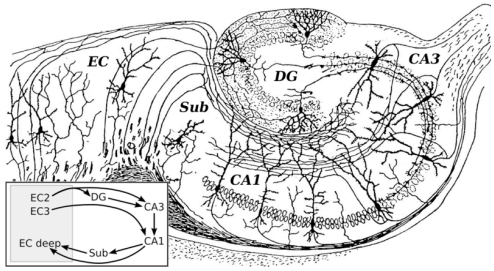
Some hints: taken from Cajal's work published more than 100 years ago. Your report may look something similar.

A. Cerebellum



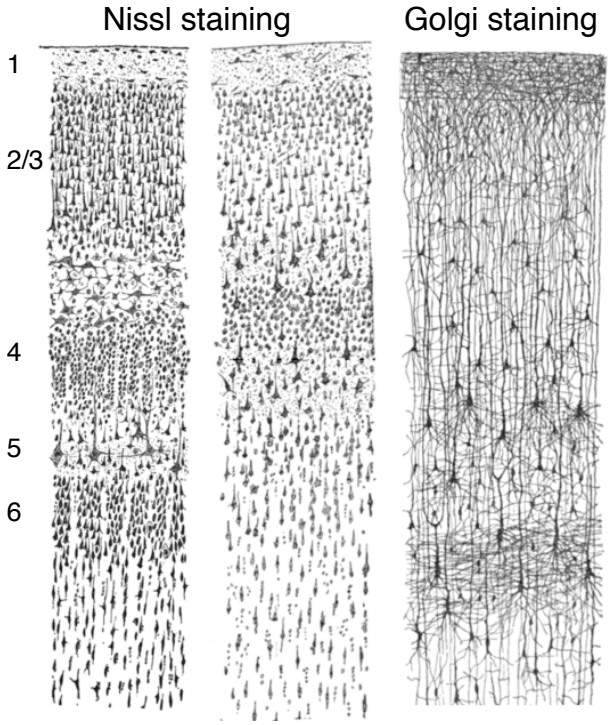
from Wikipedia Commons

B. Hippocampus



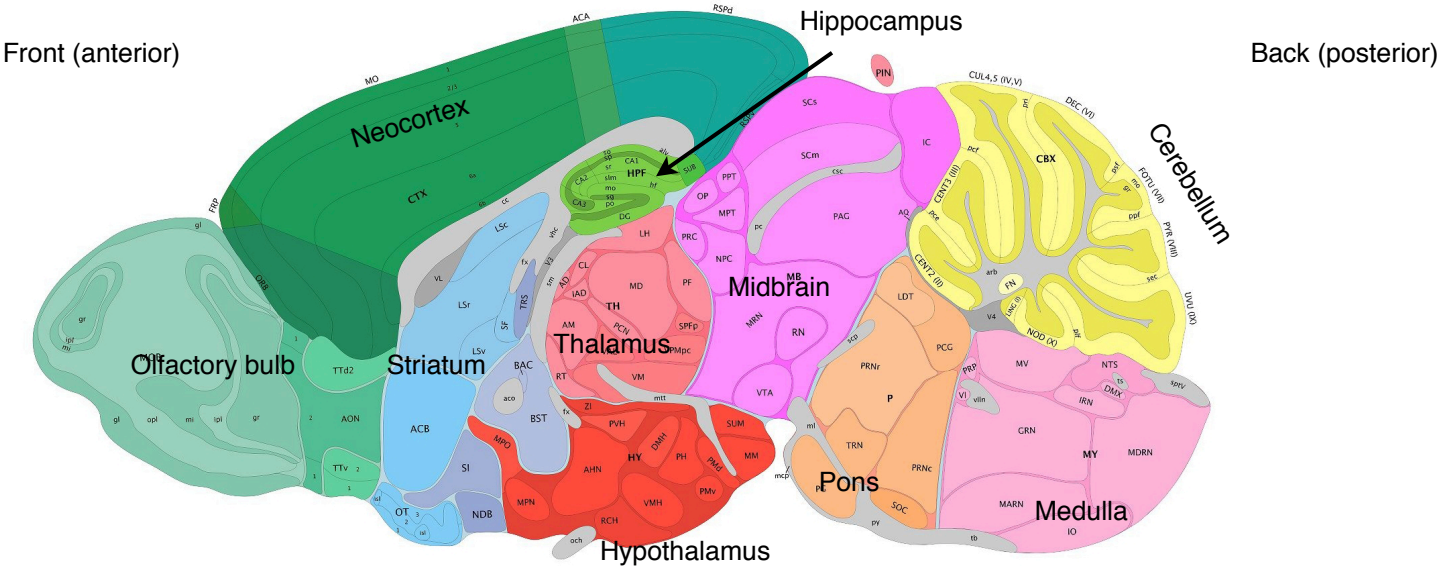
from Wikipedia Commons

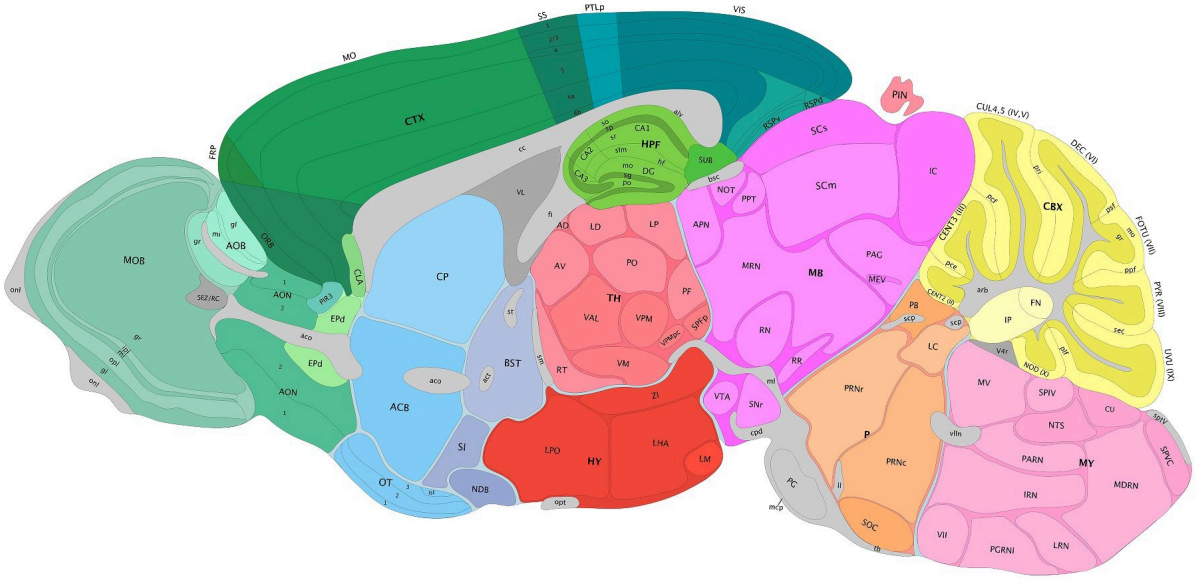
C. Neocortex



Reference atlas of the mouse brain (taken from Allen Brain Atlas)

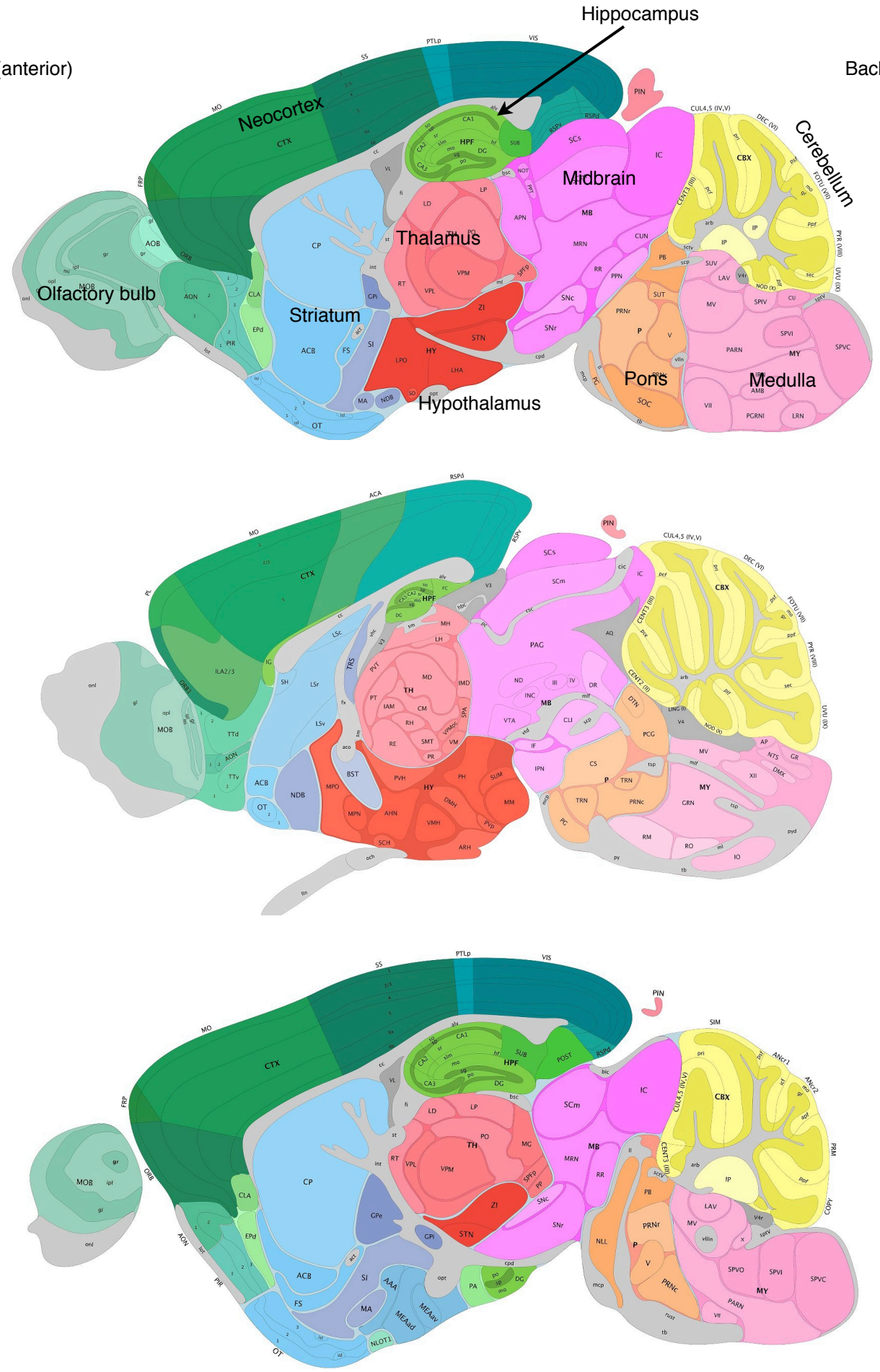
(near mid-sagittal level...most medial level)



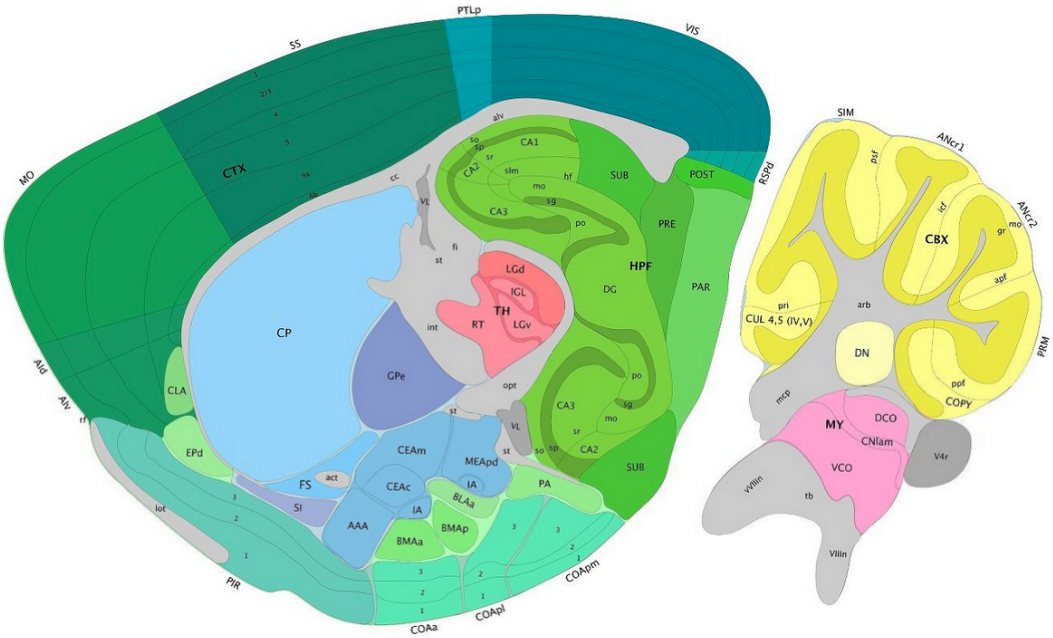


Front (anterior)

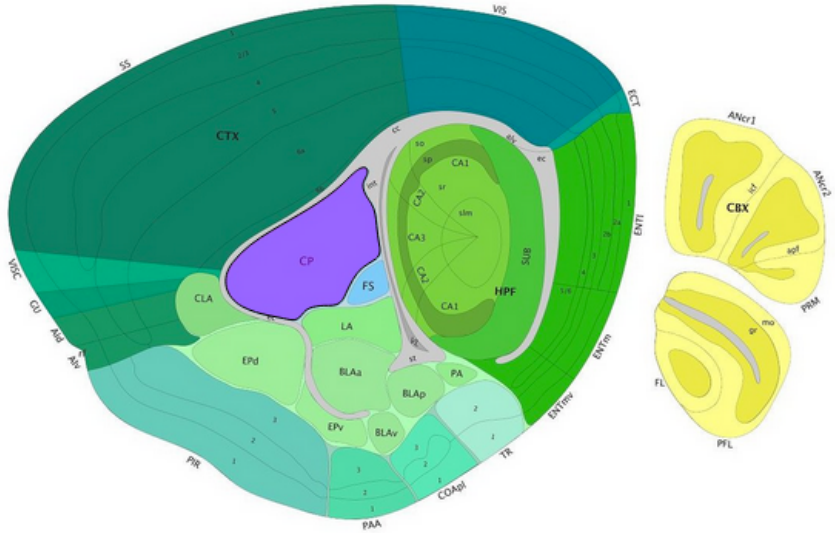
Back (posterior)



Front (anterior) Back (posterior)



(most lateral level)



LAB 4: Dissection of sheep brains

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
TURN IN YOUR REPORT FOR LAB 3

There is no report required for LAB 4, but you need to attend both LAB 4 and LAB 5 to turn in LAB 5 report.
Report of LAB 5 is due at the beginning of your LAB 6.

Objectives for LAB 4 and LAB 5:
1. Understand three-dimensional organization of the nervous system by cutting slices of sheep brains in different orientations and putting them back together

2. Prepare coronal and horizontal slices of sheep brains and perform Prussian blue staining, which will allow clear distinction of gray matter and white matter

3. Take pictures of stained slices and identify important structures

Materials:
1. Whole sheep brains submerged in water (with some dura attached; one brain per group)

2. Solutions for Prussian blue staining

Tools:
1. brain knife (one per group; be careful. It is very sharp!)
2. scissors (one per student)
3. forceps (one per student)
4. spatulas to transfer brain slices between trays (one per group)
5. blue diaper and cutting board
6. trays for staining
7. a plastic container (filled with 70% ethanol) to store stained sheep brain slices until LAB 6.
8. disposable aprons (optional)
9. gloves for each person in the group
10. iPad for taking pictures of sheep brain slices

Procedures:
Before you start:
1. Gather tools and put on aprons and gloves.

2. Fill a plastic staining box half way full with tap water. You will put the sliced sheep brains there before staining.

3. Designate one person in each group as the "timer", who will be responsible for keeping track of how long the brain slices are in each solution.

Now, let's start!
Odd-number groups will cut the brain into coronal slices and the even-numbered groups will cut them into horizontal slices. Follow the instructions below either for "Cutting coronal slices" or for "Cutting horizontal slices". To write the lab report at the end of Lab 5, you need to use printouts of photos of both coronal and horizontal slices. Coordinate with the group next to yours so each of you take pictures of both types of slices.

Cutting coronal slices:

1. Remove the dura from your sheep brain as you did in Lab 2.
2. Place the brain on a cutting board and cut it with a brain knife so that you get ~5-8mm thick **coronal slices**. Start with the most rostral part of the brain and go as far caudal as you can. Examples of slices are shown at the end of the LAB 5 manual. Display materials are also available for observation.

2. Put the slices in a plastic container with tap water and submerge them. Soak the slices in water for about 10minutes.

3. TWO REPRESENTATIVES from each group bring the plastic container to the prep room (MCB 3-141) and hand the tray to a TA. **One wears gloves and puts the lid on the container while walking on the hallway. The other student without gloves on escorts the student with the container, and opens the door to the prep room.** The TA will put the slices in heated (60°C) Mulligan solution ("Solution 1") and will incubate them for 4 minutes. Because Mulligan solution contains phenol, this step needs to be done in a fume hood to draw off the phenol fumes. Phenol selectively coats and protects myelin from the subsequent staining reaction, which turns only the gray matter into a bright blue color. As a result, a high contrast is achieved between white matter (white) and gray matter (blue).
   Mulligan's solution: 40g crystalline phenol; 5g cupric sulfate ($CuSO_4$); 1.25ml concentrated hydrochloric acid (HCl); 1,000ml distilled water

4. While you wait for the 4-minute incubation to be done, dump the water in your tray into the sink and fill it half full with tap water.

5. Once the incubation is done, collect the slices in your container and bring them back to the lab (3-146B). **Again, make sure that the person who carries the container wears gloves and puts the lid on the container while walking on the hallway.** Keep track of how long the brain slices are in water during this time (after Mulligans and before the second staining solution).

6. Using the black spatula, transfer the brain slices from your container with water into the "solution 2" tray. Let the slices incubate there for 2 minutes.
   Solution 2: 1% ferric chloride (10g $FeCl_3$ in 1,000ml distilled water)

7. Using the black spatula at the staining station, transfer the brain slices from the solution 2 tray and to your plastic tray filled with fresh tap water. Let the slices incubate in tap water for 3-5 minutes.

8. Transfer the slices to a tray with Solution 3. Incubate the slices in Solution 3 until they become dark blue. It will take less than 3 minutes.
   Solution 3: 1% potassium ferrocyanide (10g $K_4Fe(CN)_6$ in 1,000ml distilled water)

9. Transfer the slices to a tray with tap water. Leave the slices in tap water for 5 minutes, and then carefully dump half of the water down the sink drain (without also dumping out the brain slices!). Refill the tray with fresh tap water. Repeat two more times to rinse the brain slides.

10. Reconstruct the brain by putting the slices back together. Try to use major landmarks (e.g. optic chiasm) to re-assemble the slices.

11. Remove the excess water from the sheep brain slices by placing each slice on a paper towel for several seconds.

12. Place the brain slices in a plastic tray. Slices should be arranged in a rostral-to-caudal order. Make sure that the same side (rostral or caudal) of each slice is placed face up.

13. Take pictures with the iPad (provided) or your own cell phone/tablet.

14. Once you have finished cutting/staining/imaging the coronal slices, they are ready to be stored in 70% ethanol to be used in LAB 5. Transfer the slices to the brain container that you picked up at the beginning of lab, which contains 70% EtOH. Make sure you label the container with your name(s). Let the students in the next group take pictures of your slices.

Cutting horizontal slices

1. Place the brain on a cutting board and cut it with a brain knife so that you get ~5-8mm thick **horizontal slices**. Start with the most dorsal part of the brain and go as ventral as you can. Make sure you take turns within the group when you cut brain slices. Examples of slices are shown at the end of the LAB 5 manual. Display materials are also available for observation.

2. Put the slices in a plastic tray with tap water and submerge them. Soak the slices in water for about 10minutes.

3. TWO REPRESENTATIVES from each group bring the plastic container to the prep room (MCB 3-141) and hand the tray to a TA. **One wears gloves and puts the lid on the container while walking on the hallway. The other student without gloves on escorts the student with the container, and opens the door to the prep room.** The TA will put the slices in heated (60°C) Mulligan solution ("Solution 1") and will incubate them for 4 minutes. Because Mulligan solution contains phenol, this step needs to be done in a fume hood to draw off the phenol fumes. Phenol selectively coats and protects myelin from the subsequent staining reaction, which turns only the gray matter into a bright blue color. As a result, a high contrast is achieved between white matter (white) and gray matter (blue).
   Mulligan's solution: 40g crystalline phenol; 5g cupric sulfate ($CuSO_4$); 1.25ml concentrated hydrochloric acid (HCl); 1,000ml distilled water

4. While you wait for the 4-minute incubation to be done, dump the water in your tray into the sink and fill it half full with tap water.

5. Once the incubation is done, collect the slices in your container and bring them back to the lab (3-146B). **Again, make sure that the person who carries the container wears gloves and puts the lid on the container while walking on the hallway.** Keep track of how long the brain slices are in water during this time (after Mulligans and before the second staining solution).

6. Using the black spatula, transfer the brain slices from your tray with water into the "solution 2" tray. Let the slices incubate there for 2 minutes.
   Solution 2: 1% ferric chloride (10g $FeCl_2$ in 1,000ml distilled water)

7. Using the black spatula at the staining station, transfer the brain slices from the solution 2 tray and to your plastic tray filled with fresh tap water. Let the slices incubate in tap water for 3-5 minutes.

8. Transfer the slices to a tray with Solution 3. Incubate the slices in Solution 3 until they become dark blue. It will take less than 3 minutes.
   Solution 3: 1% potassium ferrocyanide (10g $K_4Fe(CN)_6$ in 1,000ml distilled water)

9. Transfer the slices to a tray with tap water. Leave the slices in tap water for 5 minutes, and then carefully dump half of the water down the sink drain (without also dumping out the brain slices!). Refill the tray with fresh tap water. Repeat two more times to rinse the brain slides.

10. Reconstruct the brain by putting the slices back together. Try to use major landmarks (e.g. optic chiasm) to re-assemble the slices.

11. Remove the excess water from the sheep brain slices by placing each slice on a paper towel for several seconds. Place the brain slices in a plastic tray. Slices should be arranged in a rostral-to-caudal order. Make sure that the same side (rostral or caudal) of each slice is placed face up.

12. Place the brain slices in a plastic tray. Slices should be arranged in a dorsal-to-ventral order. Make sure that the same side (dorsal or ventral) of each slice is placed face up.

13. Take pictures with the iPad (provided) or your own cell phone/tablet.

14. Once you have finished cutting/staining/imaging the horizontal slices, they are ready to be stored in 70% ethanol to be used in LAB 5. Transfer the slices to the brain container that you picked up at the beginning of lab, which contains 70% EtOH. Make sure you label the container with your name(s). Let the students in the next group take pictures of your slices.

LAB 5: Dissection of sheep brains

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET

You need to attend both LAB 4 and LAB 5 to turn in LAB 5 report.
Report of LAB 5 is due at the beginning of your LAB 6.

Materials:
1. Stained sheep brain slices from LAB 4 (they should be in a plastic container with 70% ethanol). Share the slices with the neighboring group so you work on both coronal and horizontal slices.

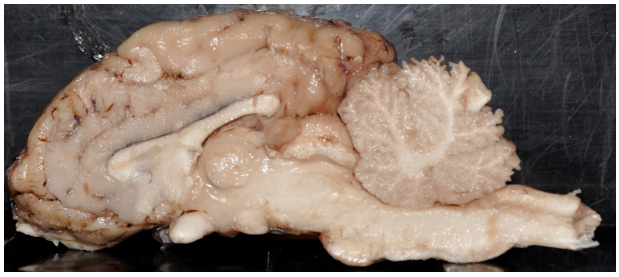
2. Printed images of the sheep brain (whole and half brains in lateral views; shown below in this page). Each student is provided with two copies of each. They will be used for the lab report.

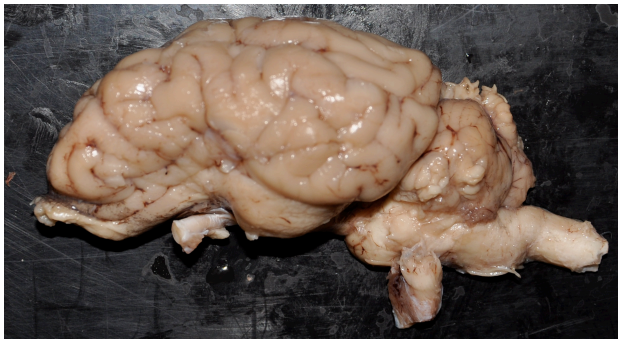
3. Printed images of coronal and horizontal sections of sheep brains (you took pictures of them in LAB 4. You will need to bring a set of printouts for each section. They should be put on your lab notebook so you can write a lab report with them.

4. Bagged slabs and plastinated slices of human brains (coronal and horizontal)

Your references:
1. Atlas at the end of this lab manual
2. Website: https://www.msu.edu/~brains/brains/sheep/index.html (bookmarked on the iPad)

Lab Report (9.0 points possible for this lab) *attendance point: 1.0, lab report point: 8.0*
Because we use photocopies of brain slices that you made after LAB 4, you do not use the carbon-copying function of the lab notebook for this report. You should have printed a copy of pictures of stained brain slices in the previous week (LAB 4). For the lab report, you need to put one copy on the original pages and turn those pages in after properly labeling them.





1. For **coronal slices**, do the following:
a. On your brain slice print out from LAB 4, number each brain slice in the anterior-to-posterior order.
b. Using both of the two printed images of sheep brains (above), draw parallel lines indicating the section planes of your coronal slices. Label each line that you draw with the number of the slice from a). **0.4 point possible**

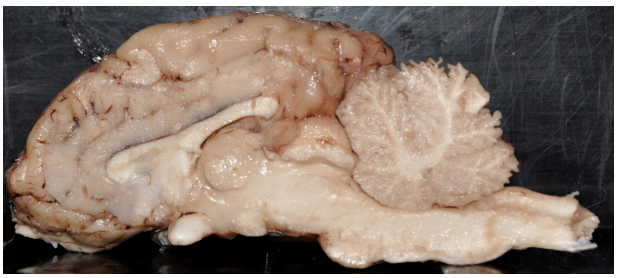
c. Identify each of the following structures and label it at least in one slice.
4.8 points possible (0.3 point for each item)

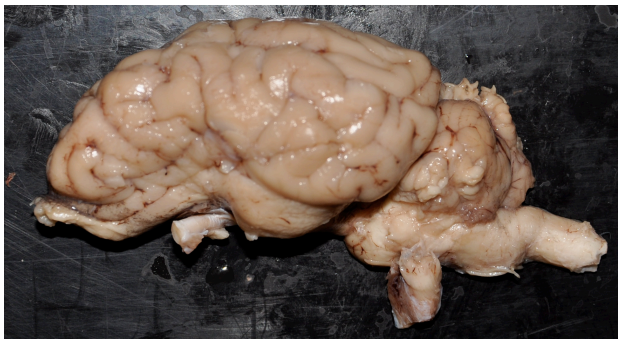
1. cerebral cortex
2. corpus callosum
3. internal capsule
4. lateral ventricle
5. thalamus
6. hypothalamus
7. third ventricle
8. optic tract
9. cerebral peduncle
10. hippocampus
11. putamen
12. optic chiasm
13. cerebral aqueduct
14. superior colliculus
15. fourth ventricle
16. cerebellum

2. <u>For horizontal slices</u>

a. On your brain slice print outs from last week, number each brain slice in the dorsal-to-ventral order.

b. Using both of the two printed images the sheep brains (below), draw parallel lines indicating the section planes of your horizontal slices. Label each line that you draw with the number of the slice from a). **0.4 point possible**

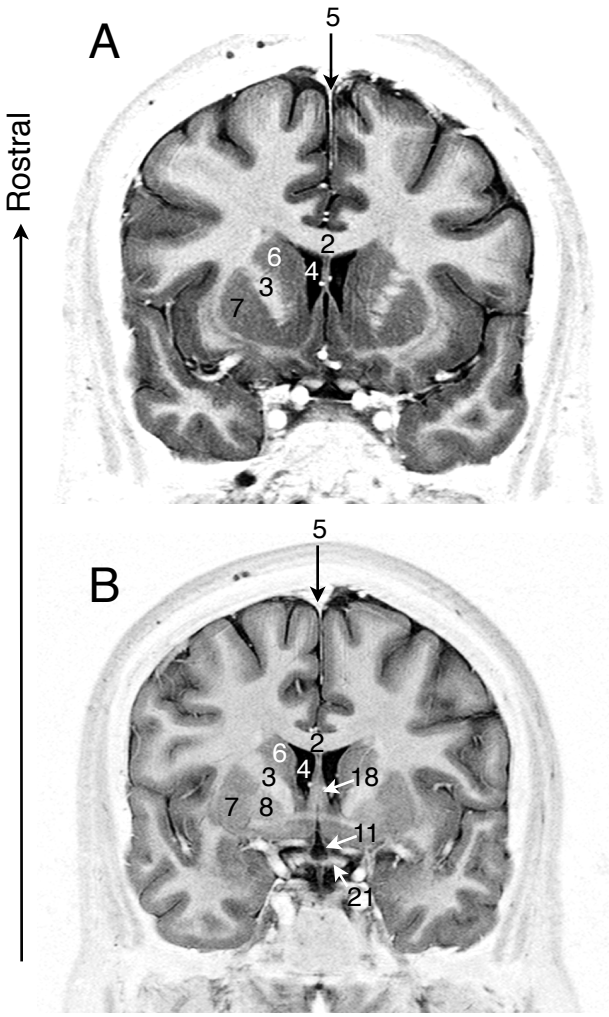


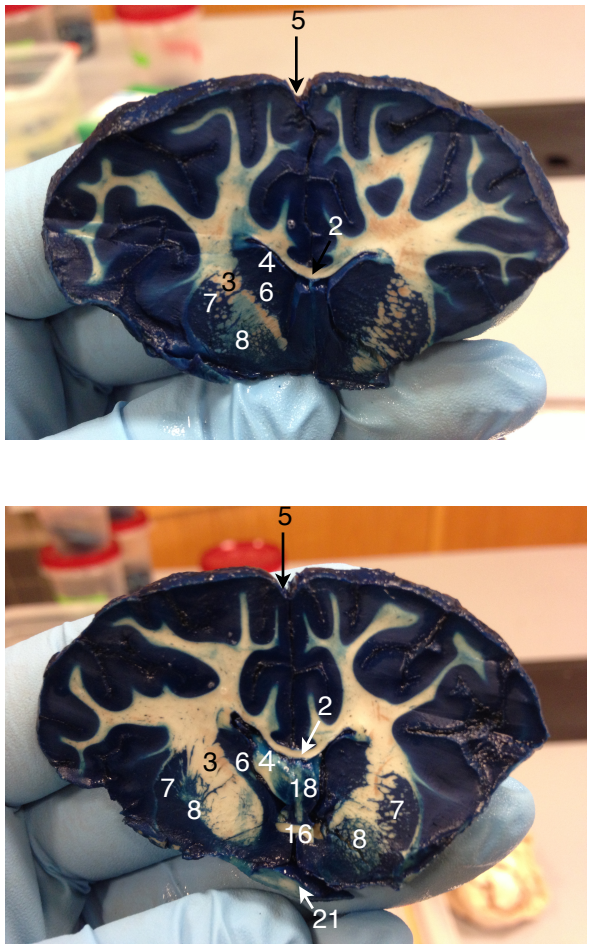


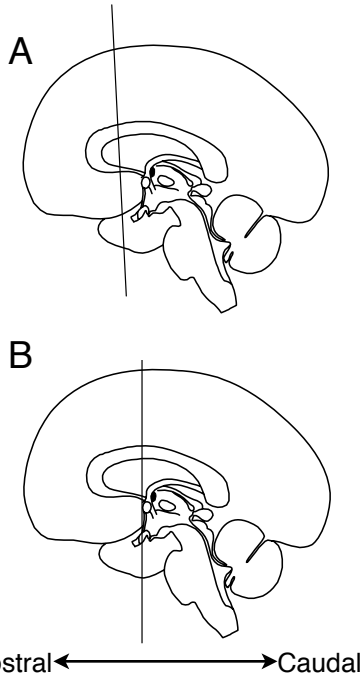
c. Identify each of the following structures and label it at least in one slice. **2.4 point possible (0.3 point for each item)**

1. internal capsule
2. corpus callosum
3. lateral ventricle
4. interhemispheric fissure (longitudinal fissure)
5. putamen
6. thalamus
7. third ventricle
8. hippocampus

Examples of coronal MRI scans of the human brain and stained coronal slices of the sheep brain

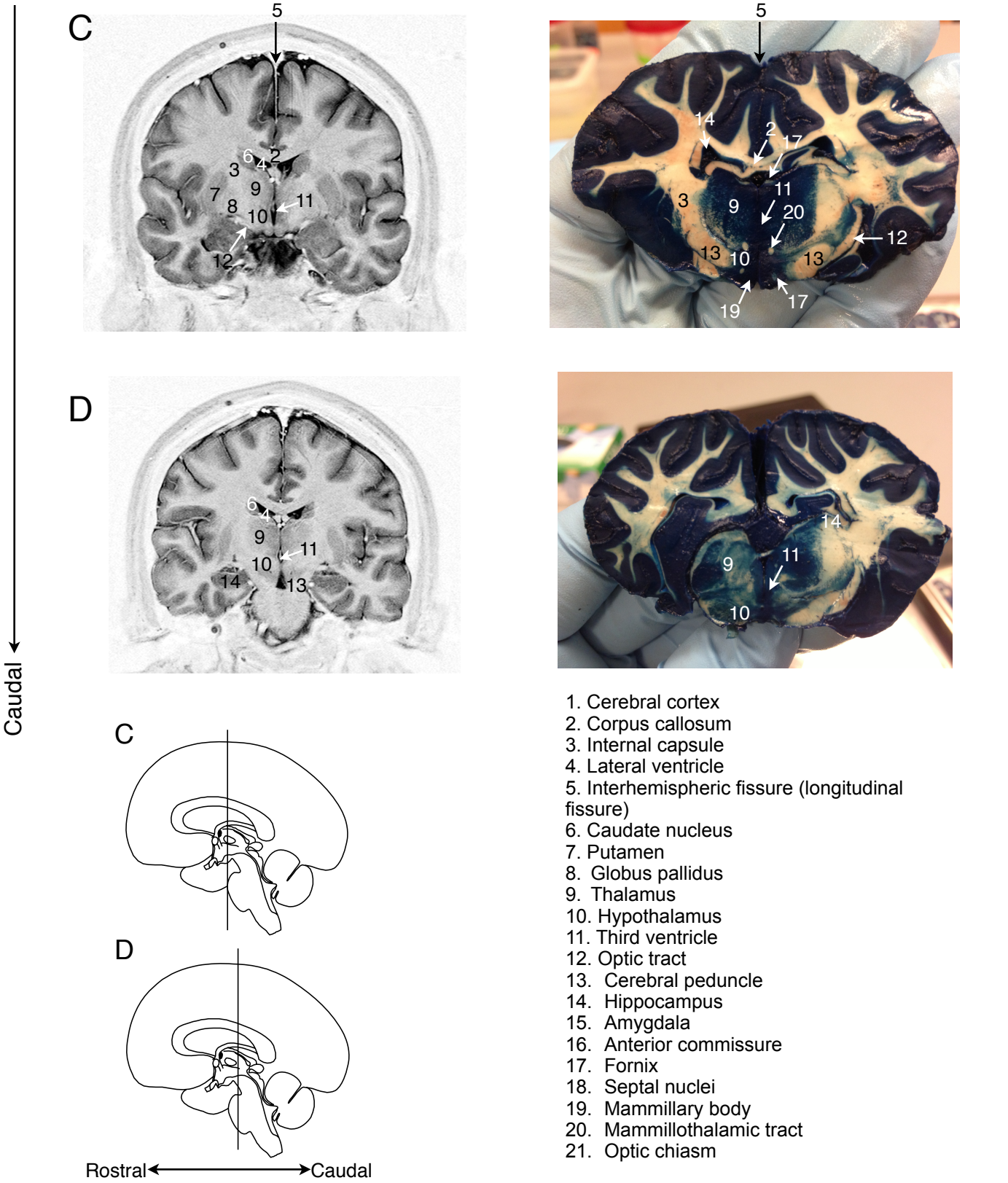






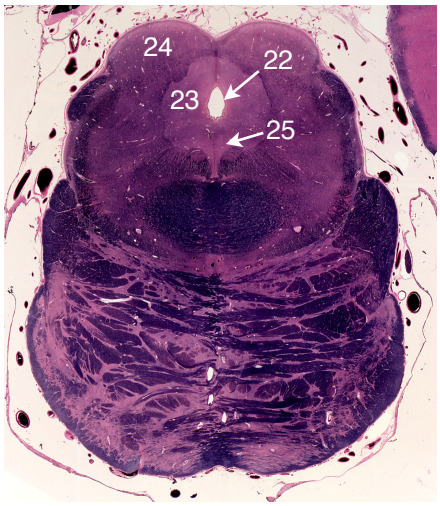
1. cerebral cortex
2. corpus callosum
3. internal capsule
4. lateral ventricle
5. interhemispheric fissure (longitudinal fissure)
6. caudate nucleus
7. putamen
8. globus pallidus
9. thalamus
10. hypothalamus
11. third ventricle
12. optic tract
13. cerebral peduncle
14. hippocampus
15. amygdala
16. anterior commissure
17. fornix
18. septal nuclei
19. mammillary body
20. mammillothalamic tract
21. optic chiasm

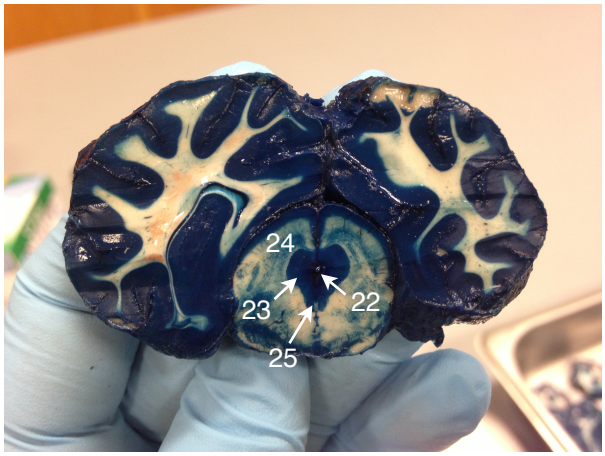
Examples of coronal MRI scans of the human brain and stained coronal slices of the sheep brain

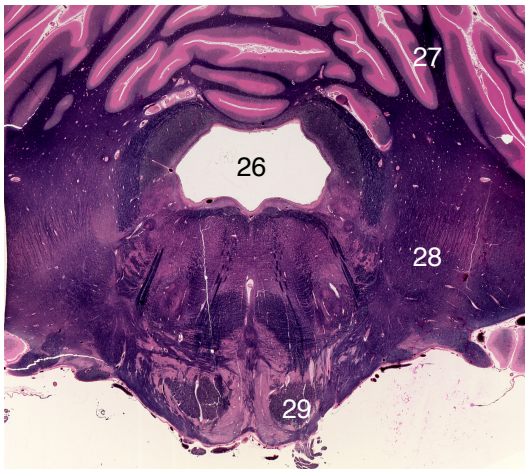


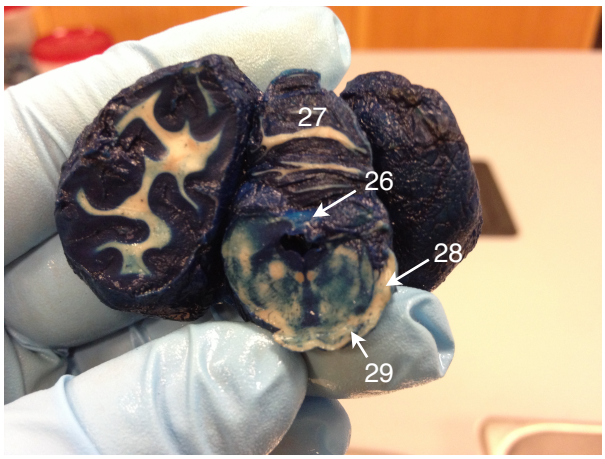
1. Cerebral cortex
2. Corpus callosum
3. Internal capsule
4. Lateral ventricle
5. Interhemispheric fissure (longitudinal fissure)
6. Caudate nucleus
7. Putamen
8. Globus pallidus
9. Thalamus
10. Hypothalamus
11. Third ventricle
12. Optic tract
13. Cerebral peduncle
14. Hippocampus
15. Amygdala
16. Anterior commissure
17. Fornix
18. Septal nuclei
19. Mammillary body
20. Mammillothalamic tract
21. Optic chiasm

Examples of stained coronal slices of the human and sheep brainstem (not to the scale)



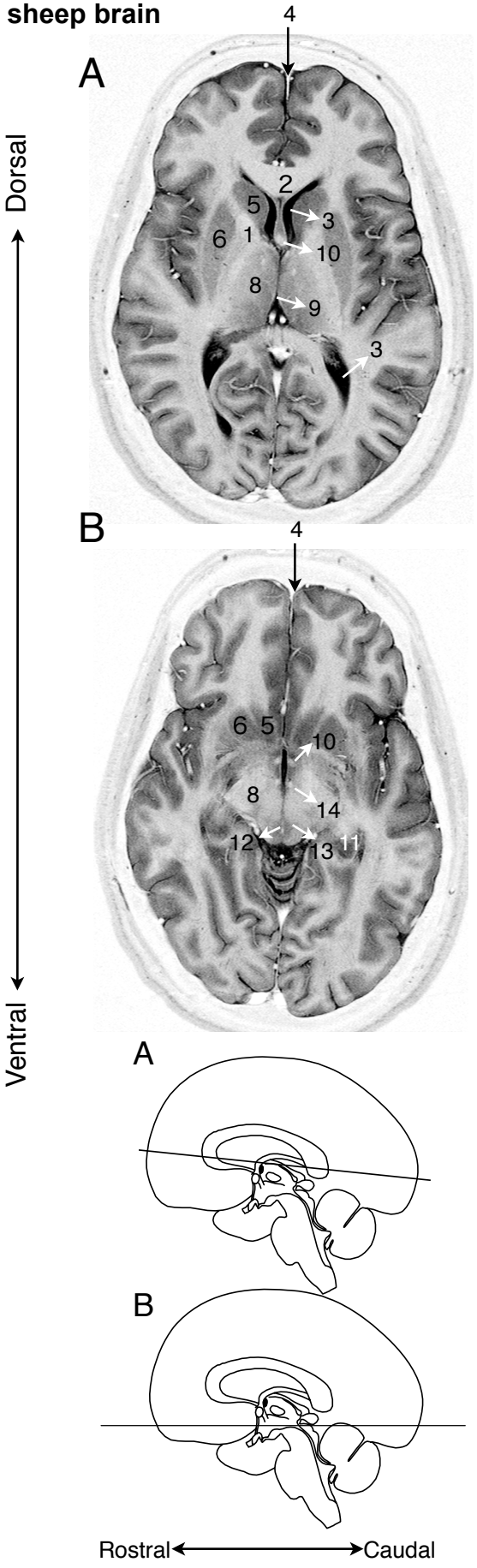


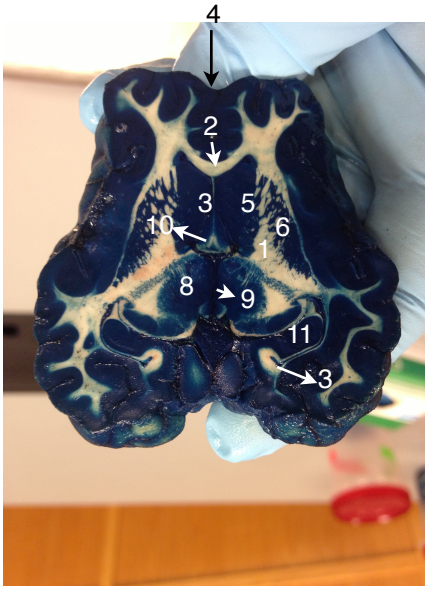


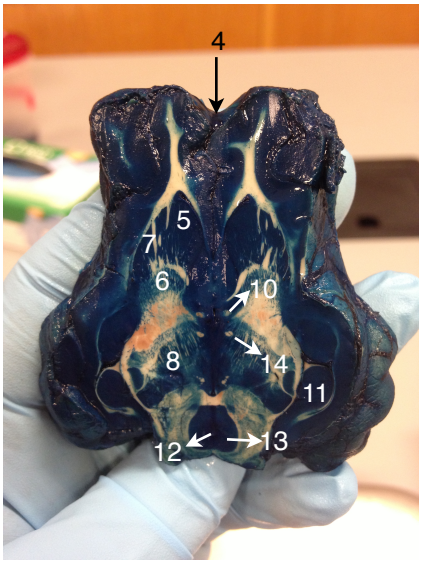


22. cerebral aqueduct
23. central gray
24. superior colliculus
25. oculomotor nucleus
26. fourth ventricle
27. cerebellum
28. middle cerebellar peduncle
29. pyramidal tract

Examples of horizontal MRI scans of the human brain and stained horizontal slices of the sheep brain







1. internal capsule
2. corpus callosum
3. lateral ventricle
4. interhemispheric fissure (longitudinal fissure)
5. caudate nucleus
6. putamen
7. globus pallidus
8. thalamus
9. third ventricle
10. fornix
11. hippocampus
12. cerebral aqueduct
13. central gray
14. mammillothalamic tract

LAB 6: Computer simulation of neuronal activity

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
TURN IN YOUR REPORT FOR LABS 4 AND 5

Report of LAB 6 is due at the beginning of your LAB 7.

Objectives for LAB 6:

1. Use computer simulations to understand some basic mechanisms responsible for generating electrical currents in neurons.

2. More specifically, learn to answer the following questions:
   -What is a membrane potential, and why is it important?
   -What components of a cell are necessary for the resting membrane potential?
   -What determines the resting membrane potential?
   -How are action potentials generated? What controls the frequency of action potential generation?

Materials:

1. Laptop computers with the ***Neurons in Action*** program (Moose and Stuart, 2007). One computer will be provided to each group.
2. Calculator that has a natural log function (your smart phone or tablet should have one) may be helpful but is not required.

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

See p.39 for details. Answer the questions in steps highlighted in blue in the manual (show your work!)

Background:

What is the resting membrane potential?

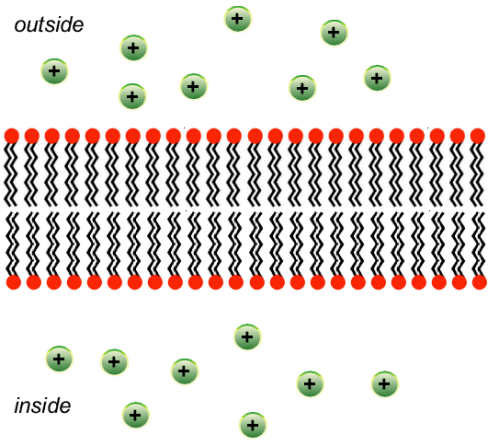
The cell membrane is made up of a lipid bilayer, which is impermeable to ions. In all cells, however, special types of proteins are inserted in the cell membrane, allowing the movement of ions between the inside and the outside of the cell. This causes a negative electric potential inside the cell. The difference in electric potential is called the voltage (unit=volt or V). At the resting state, typical neurons have a membrane potential at -60 to -70mV. This is called the resting membrane potential.

Why is the membrane potential important?

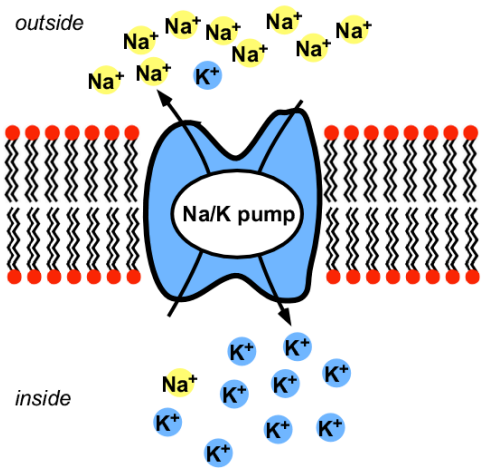
As discussed later, neurons use action potentials for much of the communication in the nervous system. For an action potential to be initiated, the membrane potential has to “depolarize” from the resting value of around -60 to -70mV to the threshold value of around −50 mV. The resting membrane potential is more negative than the threshold value for impulse generation.

What cellular components are the necessary for a resting membrane potential?

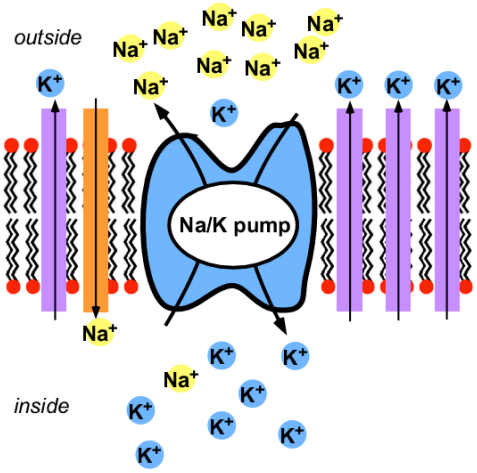
1) When the cell membrane is composed of just the lipid bilayer, there is no movement of ions across the cell membrane.



2) Na/K pump moves Na^+ ions to the outside and K^+ ions to the inside of the cell. This generates an unequal distribution of Na^+ and K^+ ions across the cell membrane.



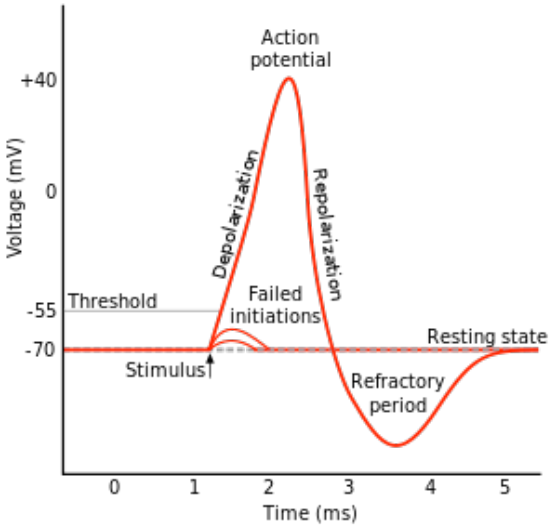
3) Further adding Na channels (orange) and potassium channels (purple) allows Na^+ and K^+ ions to move across the membrane down their concentration gradient (Na^+ moves in and K^+ moves out). In most cells, K channels are much more abundant than Na^+ channels. As K^+ ions move out of the cells via the K channels, the electric potential of the inside of the cell becomes increasingly negative, making it more difficult for K^+ ions to move out. At some point, the movement is in equilibrium, balanced between the concentration gradient and the electric potential. This results in the negative value of the resting membrane potential.



How are action potentials generated?

Excitable cells are capable of generating action potential. An action potential is a short-duration event in which the electrical membrane potential of a cell rapidly rises and falls, following a consistent trajectory. Voltage-gated ion channels are necessary for the generation of action potentials. It is important that these channels are closed when the cells are at the resting membrane potential. When the cell receives a stimulus, the membrane potential becomes less negative. This change of the membrane potential is called depolarization. When an excitatory neurotransmitter binds to its receptor, depolarization occurs as a result of the influx of positively charged ions. This is called excitatory postsynaptic potentials (EPSPs). If the membrane potential reaches a threshold, the voltage-gated Na channels open.

When the voltage-gated Na channels open, Na^+ ions rush inside the cells, causing a further rise in the membrane potential. This causes more Na channels to open, and the membrane potential becomes more positive. Then suddenly, the voltage-gated Na channels close and the potential shoots back downward, ending below the resting level. The voltage-gated K channels open at a slower time course than the Na channels, and moves the K^+ ions out of the cells, contributing to the repolarization phase of the action potential.



In this lab, we will use a computer program ***Neurons in Action (NIA2)*** to do simulations that demonstrate fundamental aspects of the resting membrane potential and the action potential. Your instructor will first demonstrate how to navigate the tutorials in NIA2. Each group of students will then work together to study the tutorials. In the lab report, you will answer some of the questions highlighted in blue color in each tutorial. To answer these questions, you will need to go through all the steps in the lab manual.

A. Start up the system

1. Click on the NIA icon (labeled ***NIA2PC***) on the desktop of the laptop computer. You will see the Firefox browser opens up the top page (Fig4.1). Click **Tutorials** to start the program (Fig4.2).

2. You will now explore two of the BASIC Level Tutorials (left column of Fig4.2).

3. As you navigate the program, you can find help in concepts and terms by clicking highlighted links.

Fig4.1

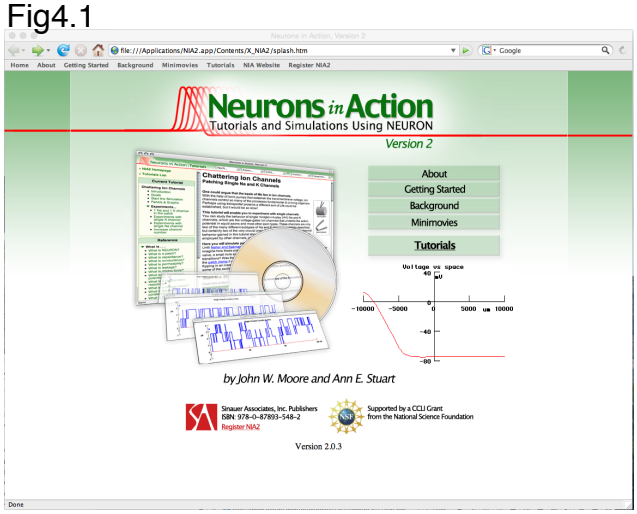
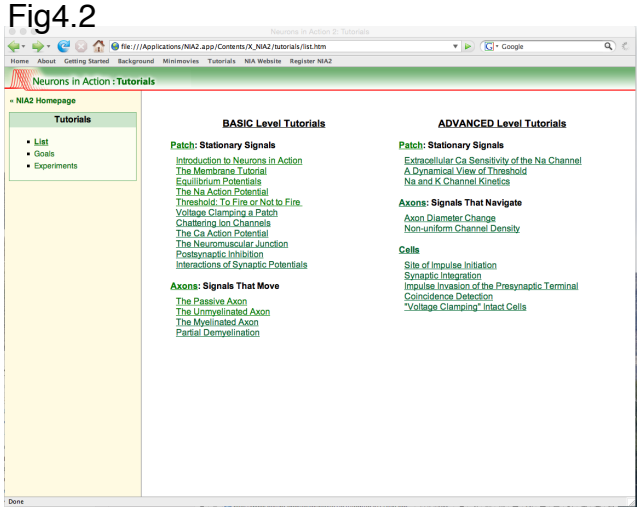


Fig4.2



B. Equilibrium Potentials

First, we will do **the Equilibrium Potential** tutorial. The equilibrium potential (or the reversal potential) is an important concept to understand the membrane potential of a cell. It is the membrane potential at which there is no net flow of a particular ion from one side of the membrane to the other. You can calculate the equilibrium potential for each ion. When a cell is permeable only to one ion, the membrane potential of that cell is the same as the equilibrium potential of that ion.

You will first simulate a hypothetical cell that is permeable only to Na^+ or K^+ ions. You will change the concentrations of ions (Na^+ and K^+) inside or outside of the cell and see how that affects the equilibrium potential. The computer program does all the simulations for you, but for those who want to know how it calculates the equilibrium potential, we provide you with the mathematical formula that allows you to calculate it with your calculator. Next, you will simulate a more realistic cell in which both Na^+ and K+ ions can move across the membrane. You will find that the membrane potential of that cell is somewhere in-between the equilibrium potentials of Na^+ and K^+ ions. The value is determined by the relative conductance of Na^+ and K^+ ions and the equilibrium potential of each of these ions.

1. Click **Equilibrium Potentials** on the left column of Fig.4.3. A new window (Fig4.4) appear. Then click "**Start the Simulation**" button (←). There will be three additional windows (Fig4.5). Click **K Conductance Only** (←) in **Panel & Graph Manager** panel. You should now see an empty graph (time-voltage).

2. In the **Patch Parameters** panel, you will see that Na chan(nel) density is zero, whereas K chan(nel) density is 1. This means that this hypothetical cell is permeable to K^+ but not Na^+.

3. Click **Run** in **Run Control** panel. You will see the BLUE graph shows that the membrane potential is about -77mV. At this voltage, movement of K^+ ions across the cell membrane is in equilibrium and there is no net movement. As shown in **Patch Parameter** panel, the intracellular K^+ concentration ($[K]_i$) is 124mM, which is much higher than extracellular K^+ concentration ($[K]_o$), 5mM. This difference in concentration is generated by the Na/K pump and drives the K^+ ion to move out of the cell down the concentration gradient. However, the negative membrane potential balances this force so that there is no net movement of K ion across the membrane. The value -77mV is exactly where this equilibrium is established. We call this the equilibrium potential (E_K) for the K^+ ion. For those of you who are comfortable with the logarithm, E_K is given in the following formula:

Fig4.3

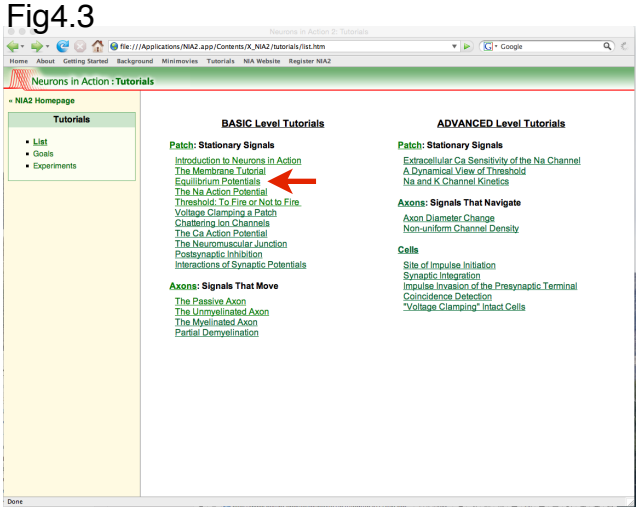


Fig4.4

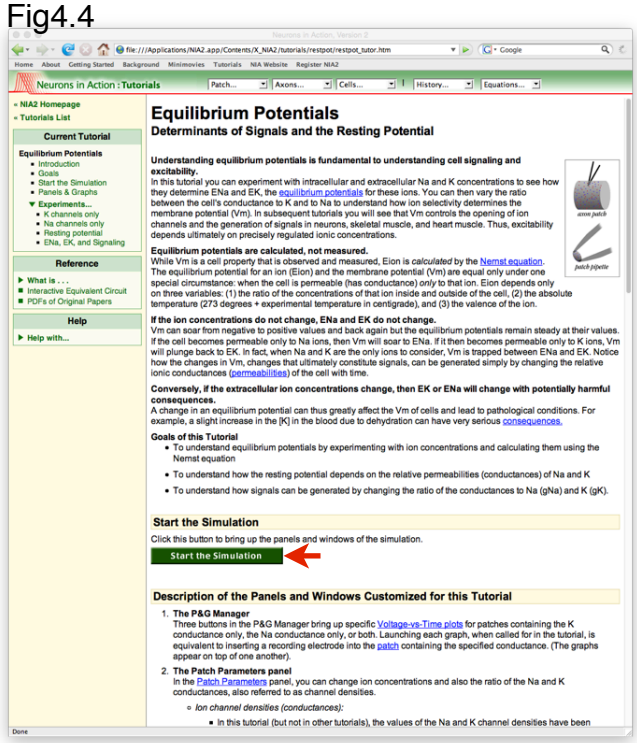
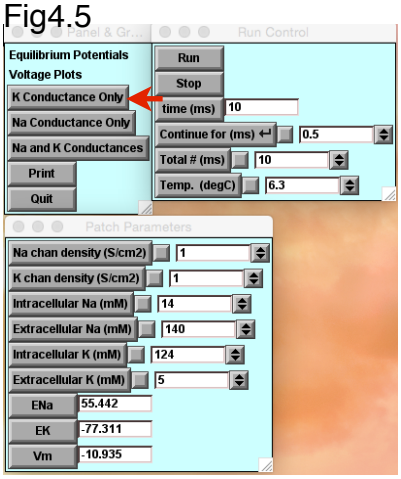


Fig4.5



Therefore, when

$[K]_o = 5mM$

$[K]_i = 124mM$

at 6.3℃ , then the formula gives

$$E_K = 2.30 \cdot 24 \cdot \log\frac{5}{124} = -77(mV)$$

The formula tells you that the equilibrium potential depends only on the K^+ concentration inside and outside the cell. This formula applies to any charged ion including Na^+ ions. In general, you can write:

$$E_{ion} = 2.30 \cdot \frac{RT}{zF} \log\frac{[ion]_o}{[ion]_i}$$ (**the Nernst equation**)

4. You can now change the $[K]_o$ in **Patch Parameters** panel and test how that changes the membrane potential. Activate the **Keep Lines** feature in the **Run Control** panel.

To save a plot for comparison with a new plot when you make changes, use the "Keep Lines" feature. Place your cursor in the plotting window, click the right mouse button, hold it down, and then select this option. If you right-click again and view the submenu, you will see a red check next to "Keep Lines," confirming your selection.

Does the membrane potential change as you expect from the formula $E_K = 2.30 \cdot 24 \cdot \log\frac{[K]_o}{[K]_i}$?

What value of $[K]_o$ will cause E_K to be zero and why?

5. Now, you can do the same simulation for Na^+ ion. Click **Na Conductance Only** in **Panel & Graph Manager** panel. Click **Run** in **Run Control** panel. You will see the RED graph shows that the membrane voltage is about +55mV, which can be calculated by the formula:

$$E_{Na} = 2.30 \cdot 24 \cdot \log\frac{[Na]_o}{[Na]_i}$$

6. A typical neuron is permeable to both K^+ and Na^+ ions (and many other charged molecules, but we will ignore that here for simplicity). Then how is the membrane potential determined? Click **Na^+ and K^+ Conductance** in **Panel & Graph Manager** panel. Click **Run** in **Run Control** panel. Measure the membrane voltage shown by the BLACK graph. Why is it at this value?

7. You should notice in **Patch Parameter** panel that the conductance (this is shown as “channel density”, because the higher the channel density is, the higher the conductance is for that ion) is 1 for both Na^+ and K^+ as default. However, in real neurons K^+ is far more permeable than Na^+. Let’s simulate this situation by changing the K channel density to 10 and then 20 while keeping the Na channel density at 1. How does that change the membrane potential?

8. The external concentrations of K^+ and Na^+ in blood and serum are very tightly regulated. Small deviations from the normal values cause serious problems and can lead to death (see below). External concentrations can become acutely abnormal, for example with the dehydration or superhydration experienced during extreme physical activity, or chronically abnormal, for example in kidney disease.

[K]out	State of the body
8.0 mM	death
7.0 mM	metabolic shock
6.5 mM	cardiac dysfunction
5.5 mM or more	defined as hyperkalemia
4.8 – 5.0 mM	normal
3.5 mM or less	defined as hypokalemia
2.5 mM or less	severe hypokalemia, cardiac arhythmia, death

[Na]out	State of the body
145 mM or more	defined as hypernatremia
140 +/-5 mM	normal
135 mM or less	defined as hyponatremia
120 mM or less	critical hyponatremia, death possible

A) A recent study (Almond et al. 2005, New England Journal of Medicine) found that out of the 488 runners who just finished the Boston Marathon in 2002, 3 had the serum (=extracellular) Na^+ concentration of 120mM or lower. Using the tutorial (**you do not need to use the formula in this manual; just enter concentrations in the NIA2 program**), find the membrane potential of the cells in these runners and compare this with the "normal value" where extracellular Na^+ is 140mM. Keep all values other than $[Na]_o$ the same between the marathon runners and the controls: channel density: Na=1, K=20, temperature: 37℃. What did you find?

B) A person with renal failure may have the extracellular K^+ concentration as high as 8mM. What happens to this person's resting membrane potential compared with the membrane potential of a normal person with the extracellular K^+ concentration at 5mM. Again, keep all the values other than $[K]_o$ unchanged from the simulation above (the marathon runner case). In which case do you see more drastic changes in the membrane potential? Why?

9. The resting membrane potential is given in the following formula:

$$E = 2.30 \cdot \frac{RT}{zF} \log \frac{P_{Na}[Na]_o + P_K[K]_o}{P_{Na}[Na]_i + P_K[K]_i}$$

P_{Na} and P_K represents conductance of each ion (channel density in the tutorial). This formula explains why the resting membrane potential (E) is somewhere between E_K and E_{Na}.

10. Finally, what if the conductance ratio for Na^+ and K^+ is reversed (e.g., 100 for Na^+ and 1 for K^+)? Will that change the membrane voltage? Find it out using the NIA2 program. This is what happens when an action potential is generated. You will look at this in more detail in the next tutorial, **Sodium Action Potential**.

C. The Sodium Action Potential

In this tutorial, you will simulate a cell that is equipped with 1) cellular components required to form resting the membrane potential (same as the one used in **Equilibrium Potential** tutorial) and 2) voltage-gated Na and K channels, which are required for the cell to fire action potentials. In a real neuron, the depolarization that triggers the action potential is provided by excitatory postsynaptic potential (EPSP). In this case, binding of a neurotransmitter to its receptor on the cell membrane causes a movement of positive ions into the cell.

In this tutorial,
-you inject current into the cell and analyze how action potentials are generated
-you observe the underlying current and conductance changes during the action potential
-you change stimulus parameters such as the amplitude of the injected current and observe how that affects the action potential
-you simulate the use of anesthetic agents and toxins, which change the conductance of Na^+ and/or K^+ ions
-you examine the refractory period following the action potentials and its consequences for membrane excitability

1. Click **The Na Action Potentials** on the left column of Fig4.6. When you click "**Start the Simulation**" button, three panels will appear (Fig4.7). You will also see an empty graph on the right.

2. Click **Voltage vs Time Plot** in **Panel & Graph Manager** panel and then **Reset & Run** in **Run Control** panel. You should see a BLACK graph (time-voltage) as well as RED (ENa: equilibrium potential for Na^+) and BLUE (EK: equilibrium potential for K^+) graphs (Fig4.8). The GREEN graph show the brief stimulating current that triggers the depolarization. The black one shows the action potential.

Fig4.6

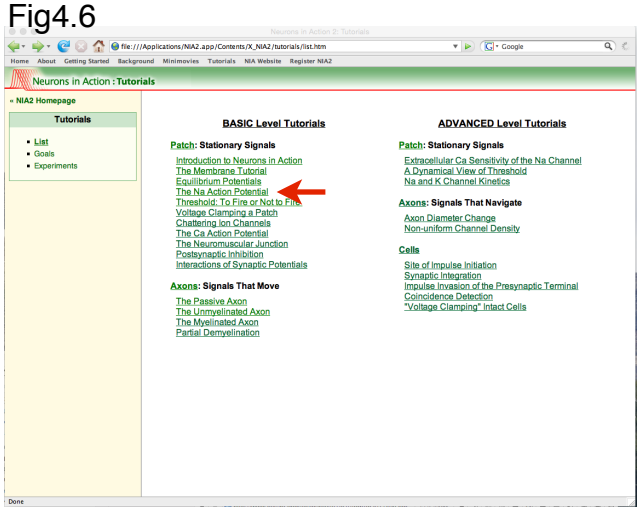


Fig4.7

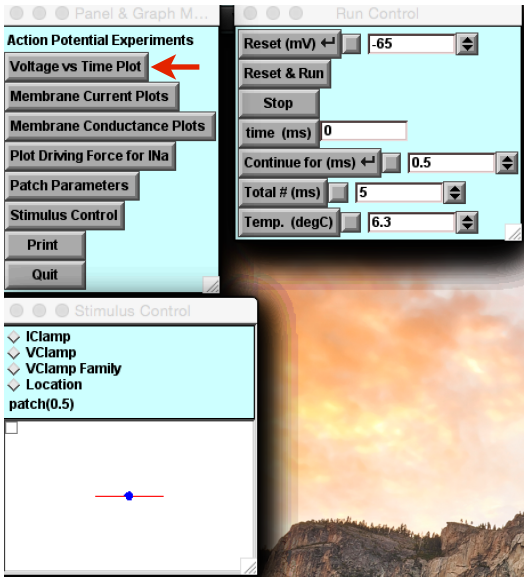
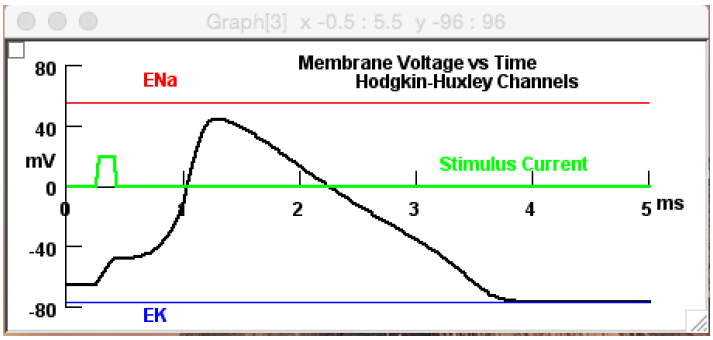
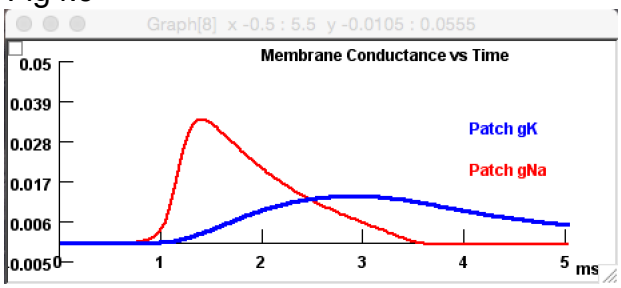


Fig4.8



3. Plot the conductance of Na^+ and K^+ during the action potential. Click **Membrane Conductance Plots** in **Panel & Graph Manager** panel and then **Reset & Run** in **Run Control** panel (Fig4.9). You will see that the Na^+ conductance rises very quickly because the voltage-gated Na channels are open at a negative membrane voltage. K^+ conductance increases with a delay because they open open when the voltage is positive.

Fig4.9



4. Now you are going to change the stimulus condition and then the conductance of Na^+ and/or K^+ ions. Observe the effects on the membrane potential (voltage), currents and ion conductance.

5. Change the amplitude of stimulus current.
Click **IClamp** in **Stimulus Control** panel. You will see that in the default mode, the amplitude of the stimulus current is 0.2nA. Change this to a progressively lower value (0.15nA, 0.10nA, 0.08nA, 0.07nA, 0.06nA, 0.05nA). Click **Reset & Run** in **Run Control** panel every time you change the value. Activate the **Keep Lines** feature in the **Run Control** panel so you can compare the curves in the same graph. What happens to the action potential and conductance and why? You can also change duration of the current and see if there is also a threshold for generating an action potential.

6. Change the conductance of Na^+ ions. Next, let's simulate the blocking the Na channels by tetrodotoxin (TTX). TTX is found in the liver of the pufferfish and is a highly specific blocker of Na channels. Click **Patch Parameters** in **Panel & Graph Manager** panel and decrease the Na channel density stepwise from the default value of 0.12 to progressively lower values (0.10, 0.09, 0.06, 0.04, 0.03, 0.02...) and click **Reset & Run** in **Run Control** panel each time you change the value. What do you see? At which value of channel density, do you stop seeing an action potential?

7. Change the conductance of both Na^+ and K^+ ions. Finally, simulate the effects of anesthetic agents like procaine and lidocaine. They reduce both the Na^+ and K^+ conductances by almost equal factors. Reduce the values of both conductances (the Na channel density and the K channel density) by a factor of two at a time. Compare action potentials generated at different "concentrations" of anesthetic by continuing to divide by a factor of two. By how much must you reduce the two conductances to block the generation of the action potential? Which is more effective at blocking action potentials, a toxin that selectively blocks Na channels or the anesthetics that block both Na and K channels? Why? (Hint: the Na^+ and K^+ currents compete in trying to depolarize and re-polarize the membrane)

(Optional exercises)

8. Refractory period (Na channel inactivation). You will test how soon after an action potential the neuron can fire another action potential. To do this, you first increase the recording time by changing **Total #** (ms) on **Run Control** panel from 5 to 20. Click **Stimulus Control** in **Panel & Graph Manager** panel and get another **Stimulus Control** window so you can control two current stimuli. You give the first stimulus with no delays (set the **Delay** at 0ms). For the second stimulus, you first set the delay at 9ms. Click **Reset & Run** in **Run Control** panel and see if the second action potential is generated. Change the delay period and determine how long it should be to evoke the second action potential?

9. Next, let's probe the refractoriness following the spike by decreasing the delay of the second stimulus to 11, 10, 9, 8, 6, 5, and 4 ms and finding the threshold amplitude of the stimulus current (necessary to evoke the second action potential) at each of these times. You can change the amplitude in the **IClamp** panel of the second stimulus. Do you need higher and higher current amplitude to evoke the second action potential as you decrease the time between the two stimuli?

10. If you give the second stimulus too soon after the first, the second stimulus will not lead to the second action potential. This is called the refractory period and is due to the inactivation of voltage-gated sodium channels. What do you think is the effect of the refractory period?

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*

Answer the questions in steps highlighted in blue (show your work!)

1. For Equilibrium Potential Tutorial, answer the questions in step 8 (2 points possible).

2. For the Na Action Potential Tutorial, answer the questions in step 6 (1 point possible) and step 7 (1 point possible).

LAB 7: SOMATOSENSORY SYSTEM

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
NEXT, TURN IN YOUR REPORT FOR LAB 6

Report of LAB 7 is due at the beginning of your LAB 8.

Objectives:
1. Review the somatosensory pathways from the peripheral nerves to the somatosensory cortex. Note that two separate pathways (pain/temperature sensation and touch/proprioception) merge in the thalamus
2. Identify structures of the somatosensory pathways by observing whole brain/spinal cord and slides from human materials.
3. Understand the differences in the sizes of the receptive fields for different parts of the body surface.
4. Use statistical methods to determine whether the values in two different samples are significantly different from each other.

Materials:
1. Human brains (whole, half) and spinal cord (one set per group; keep them moist at all times)
2. Stained slides of human brain and spinal cord

Weigert (myelin) and cresyl violet (Nissl substances/ribosome in the cell body) stained slides will be used to identify the structures involved in somatosensory system.

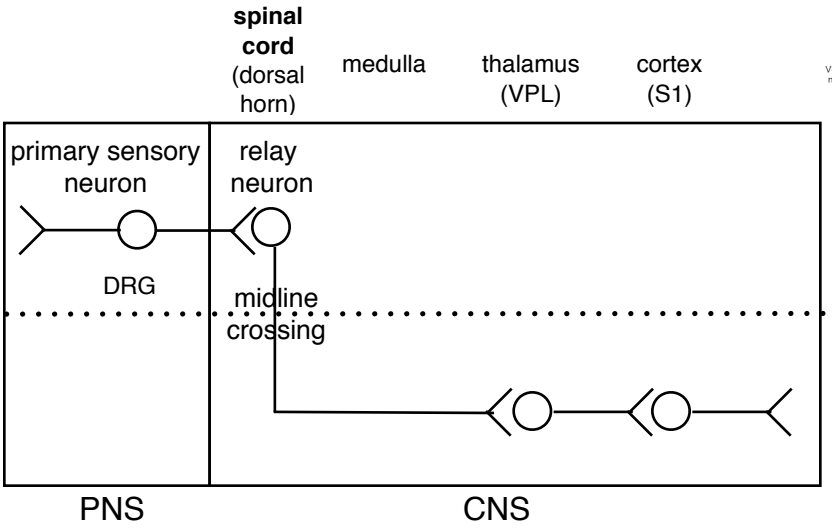
3. Tools for the two-point discrimination test (eraser with pins)

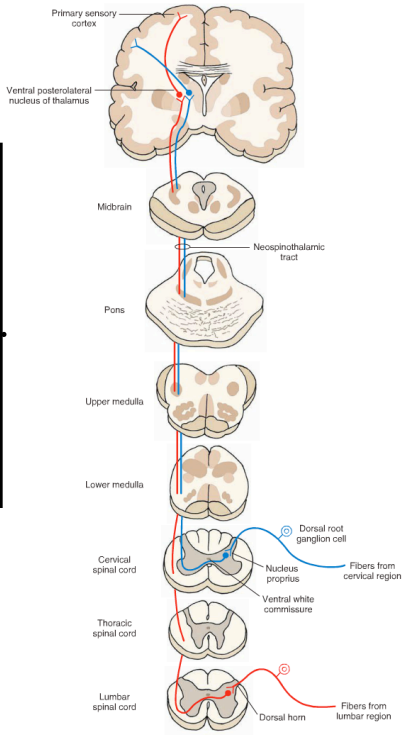
Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)
1. Report on two-point discrimination experiments (2.5 points possible)
2. Answer clinical questions (1.5 points possible)

Overview of central somatosensory pathways:

There are two pathways (pain/temperature vs touch/proprioception) that convey somatic sensation. They take separate paths in the CNS until they reach the thalamus. The pathway from the thalamus to the somatosensory cortex is shared.

1) Pain/temperature for the body

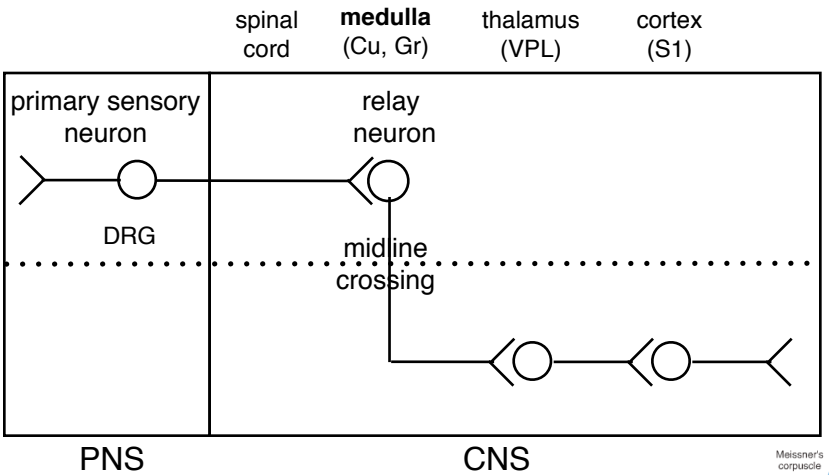


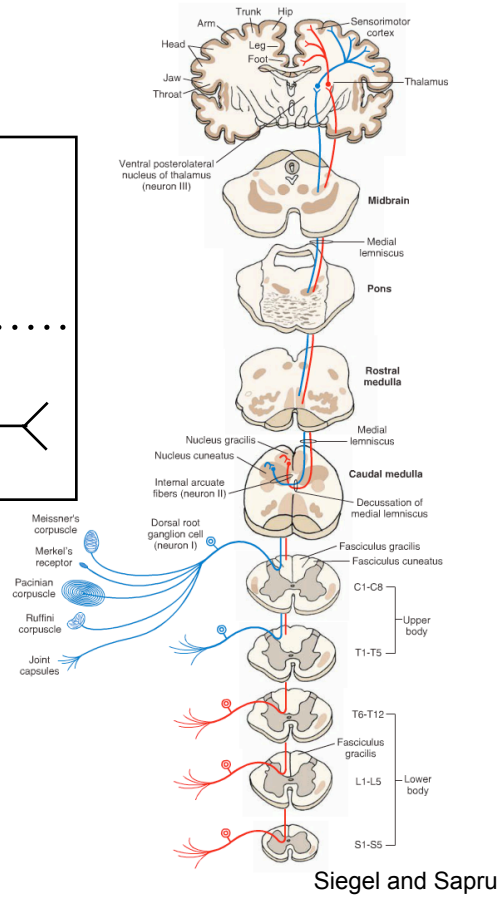


dorsal horn → thalamus via **spinothalamic tract**

VPL: ventral posteriolateral nucleus
S1: primary somatosensory area (postcentral gyrus)

2) Touch/proprioception pathway for the body



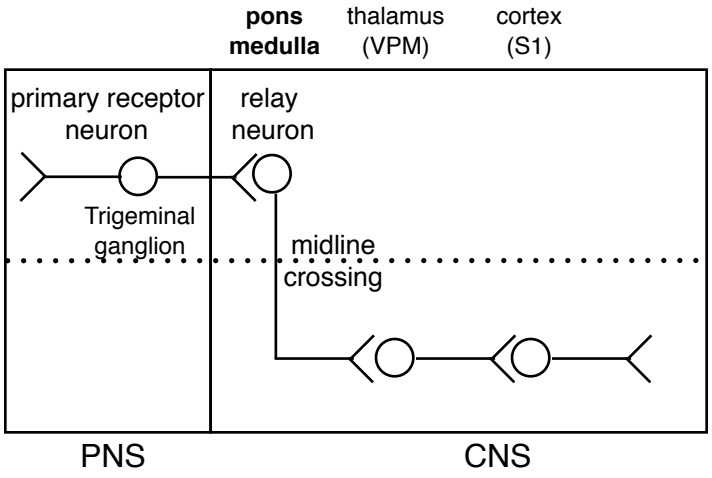


medulla → thalamus via **medial lemniscus**

Cu: cuneate nucleus (for upper body)
Gr: gracile nucleus (for lower body)
VPL: ventral posteriolateral nucleus
S1: primary somatosensory area

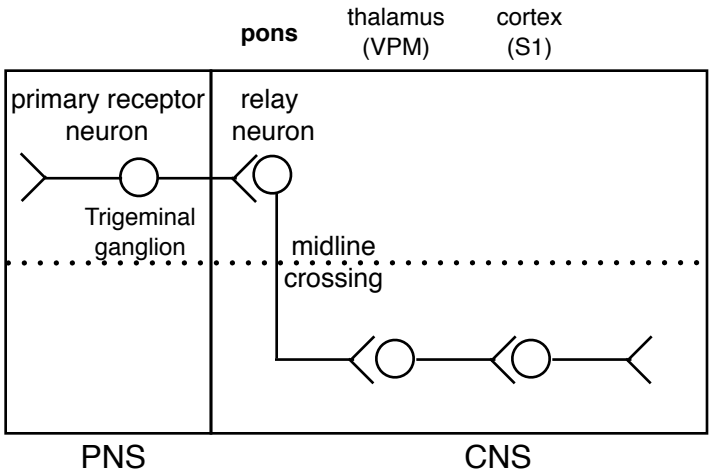
Siegel and Sapru

3) Pain/temperature pathway for the face



Relay neurons are located in a thin, long nucleus spanning from the pons to the spinal cord (spinal trigeminal nucleus)
VPM: ventral posteriomedial nucleus
S1: primary somatosensory area

4) Touch/proprioception pathway for the face

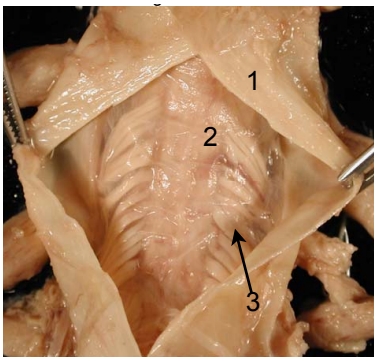


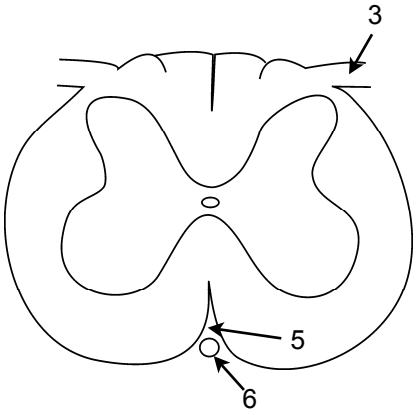
Procedure

1. Observation of gross materials (<u>you do not write a lab report on this, but you will be asked to identify the labeled structures in exams</u>)

<u>Human spinal cord</u>

Identify the following structures



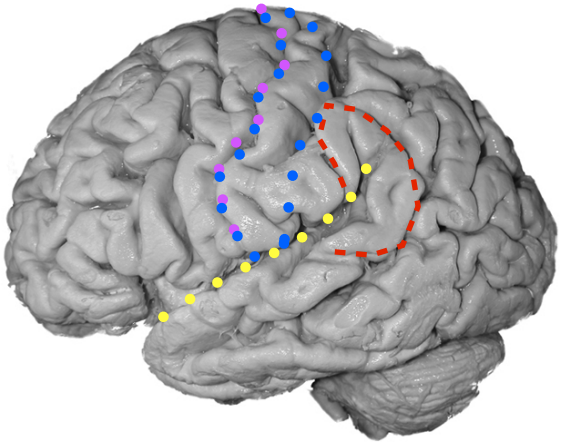


<u>review of the meninges</u>
1. dura mater
2. arachnoid (shiny covering of the spinal cord)

<u>spinal cord structures</u>
3. dorsal roots (sensory axons)
4. ventral roots (motor axons)
5. anterior median fissure
6. anterior spinal artery

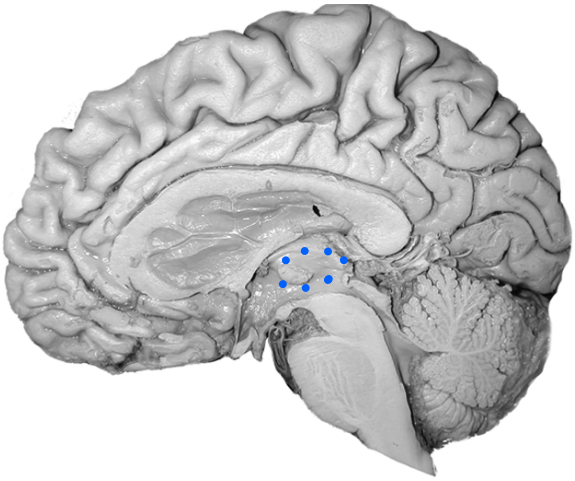
(5 and 6 are good landmark for the ventral side of the spinal cord)

<u>Human brain</u>



Identify the primary somatosensory cortex (in the whole brain) and the thalamus (in the half brain)

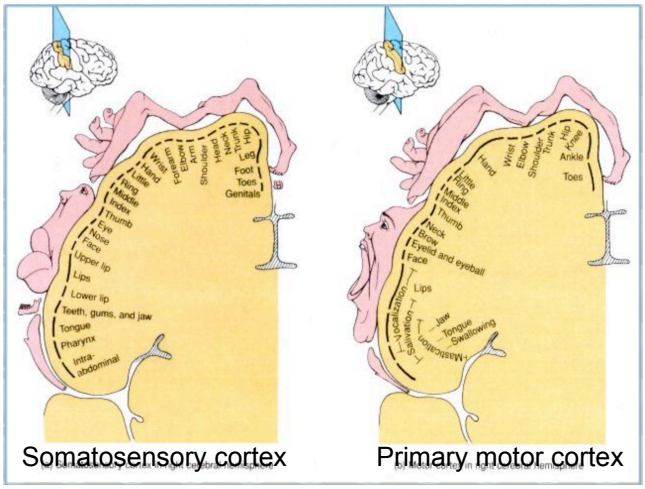
In the lateral view, follow the lateral sulcus (yellow line) caudally, and you will find a gyrus that caps the dorsal end of the lateral sulcus (outlined by the red dotted line). Identify the gyrus that is immediately rostral to this gyrus. This will be the post central gyrus that includes the **primary somatosensory cortex** (outlined by the blue dotted line). This gyrus is rostrally bounded by the central sulcus (purple dotted line)



The ventral posterior nucleus (including both VPL and VPM, which are not visible from the medial surface of the brain) is one of many nuclei (neuronal groups) within the thalamus. Identify the thalamus as a whole on the medial side of the half brain (outlined by a blue line)

There is a topography present at every level of the sensory pathways (visual, somatosensory, auditory)

Sensory homunculus (left) in the cerebral cortex



(originally from Penfield and Rasmussen (1950)

Medial/dorsal: leg, genitalia

Lateral/ventral: face, tongue

2. Observation of histological slides (<u>you may be asked to identify these on exams</u>)

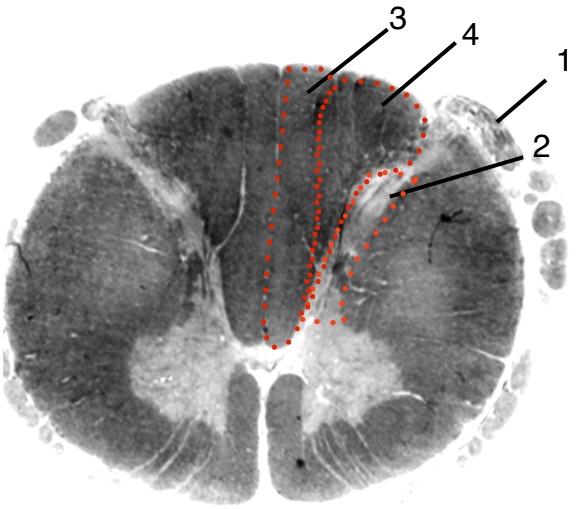
-Weigert (myelin) stained human spinal cord sections

-Weigert and cresyl violet (Nissl substance/ribosomes in the cell body) stained human brainstem slides

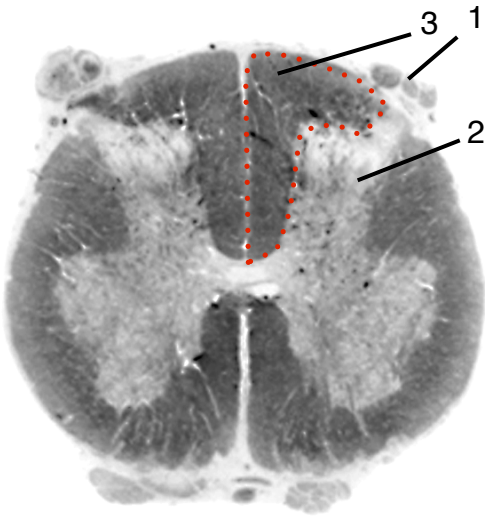
Identify the following structures that are related to the somatosensory system.

<u>spinal cord</u>

1. dorsal roots
2. dorsal horn
3. gracile fasciculus (tract of sensory axons that reach the gracile nucleus in the medulla)...touch/proprioception for the lower body
4. cuneate fasciculus (tract of sensory axons that reach the cuneate nucleus in the medulla)...touch/proprioception for the upper body

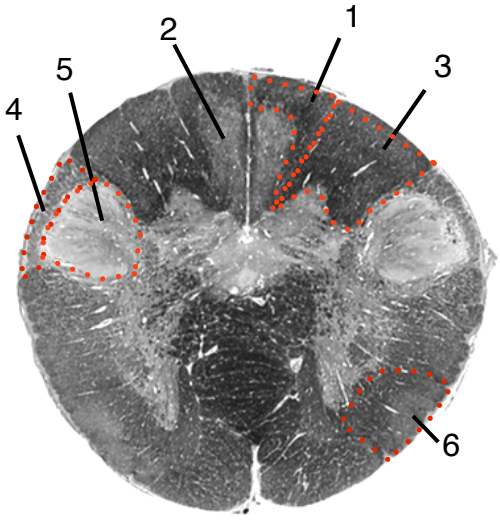


Slide 54 (cervical spinal cord)



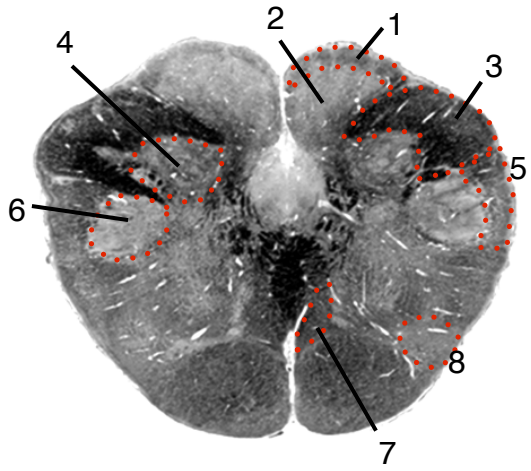
Slide 56 (lumbar spinal cord)

<u>brainstem (medulla)</u>



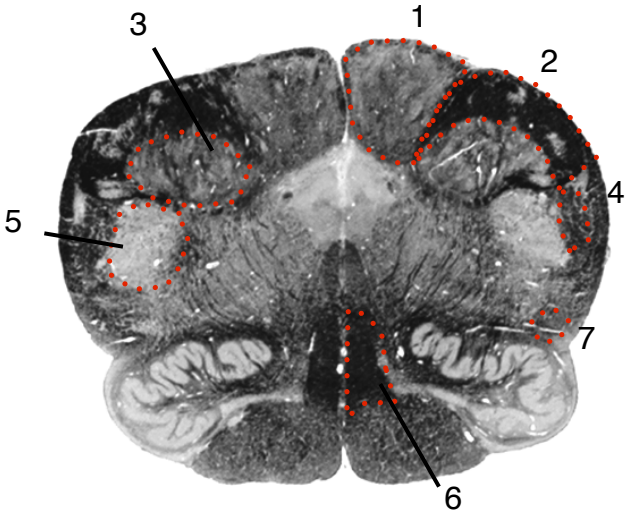
Slide 2-4 (caudal-most medulla)

1. gracile fasciculus
2. gracile nucleus
3. cuneate fasciculus
4. spinal trigeminal tract (tract of sensory axons that reach the spinal trigeminal nucleus)...pain/temperature for the face
5. spinal trigeminal nucleus
6. spinothalamic tract



1. gracile fasciculus
2. gracile nucleus
3. cuneate fasciculus
4. cuneate nucleus
5. spinal trigeminal tract
6. spinal trigeminal nucleus
7. medial lemniscus (tract of sensory axons that reach the VPL nucleus of the thalamus)...touch/ proprioception for the body)
8. spinothalamic tract

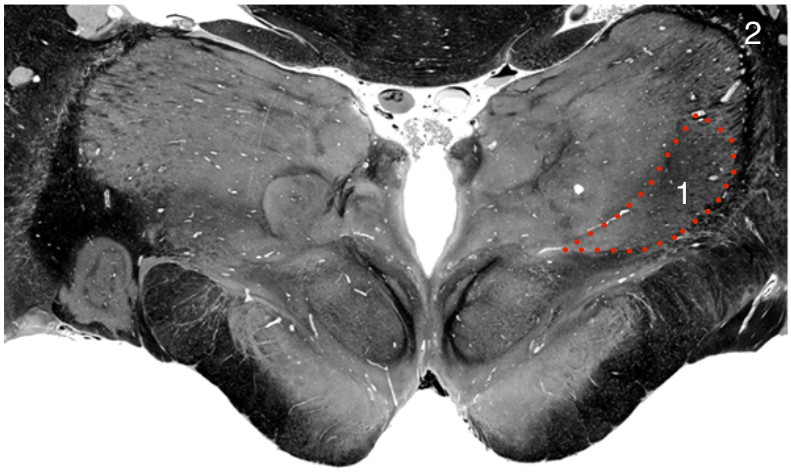
Slide 5 (medulla)



1. gracile nucleus
2. cuneate fasciculus
3. cuneate nucleus
4. spinal trigeminal tract
5. spinal trigeminal nucleus
6. medial lemniscus
7. spinothalamic tract

Slide 6 (medulla)

<u>diencephalon</u>



1. ventral posterior nucleus of the thalamus (VPL and VPM)
2. internal capsule

Slide 19-20 (diencephalon)

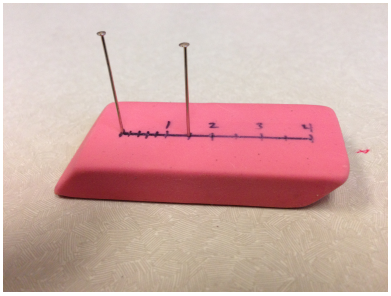
2. Two-point discrimination test (2.5 points possible for the lab report).

-With this experiment, you will explore the size of the receptive field on the skin in different parts of your body.

-Pair with another student. One becomes the subject and the other becomes the tester. After the experiment, switch the roles so you become both the subject and the tester.

-The subject closes his/her eyes.

-The tester uses a plastic eraser with a ruler drawn on it (shown in the picture on the right) and places two pins at a defined distance from each other.



-The tester presents either a single pin or two pins on the skin of four different body parts (index finger, upper arm, cheek and back of the neck) of the subject. For each stimulus, the subject tells the tester if the stimulus is single or paired. To ensure a valid result, at least one-third of all trials should use a single pin. Stimulate only the right side or the left side of the back of the neck when you use two pins.

-For each of the four body parts, the tester determines the smallest distance between the two pins at which the subject is able to tell that the stimulus is paired, not single, with at least 80% of accuracy. To determine this, at least 10 trials with two pins should be done for each distance. This value will be the subject's threshold of discrimination for the given body part. **Write down the threshold value (in mm) on the subject's lab notebook (use a table below). Filling this first column of the table in your lab report is worth <u>0.5 point</u>.**

-Next, you will combine the data for all the students in the room to find out the mean and standard deviation of the measured threshold values for each body part. See next page for a brief background of statistics. Using the iPad, open the Google spreadsheet and enter your numbers as the subject (in mm). There will a subject # column on the Google spreadsheet. Indicate the that number on left upper box in the table below ("your subject #").

-In the same table below, write down the mean and standard deviation of threshold values for the entire group (second and third empty columns, respectively). Filing these two columns is worth <u>0.5 point</u>. See a brief background for statistical analysis at the end of this lab manual.

your subject #=	**your own threshold as the subject (mm)**	**mean threshold for the whole section (mm)**	**standard deviation (mm)**
Body part	*(this column=0.5 point)*	*(these two columns=0.5 point)*	
index finger			
upper arm			
cheek			
back of neck			

-In your lab report, describe which body part shows the smallest mean of threshold and which one shows the largest, and discuss what this result tells you about the distribution of sensory receptors on the skin (0.5 point possible).

-Find the p-value for the comparison between upper arm and back of the neck. The value is automatically calculated on the bottom of the Google spreadsheet. Is there a statistically significant difference between the threshold for these two body parts? This is worth 0.5 point in the lab report.

-Finally, discuss what would cause variability of threshold values between subjects? Explain in one sentence (0.5 point possible)

Note:

1. *You will receive an email on sharing the data template on Google drive. Just click on the Google Drive icon and you will see a list of spreadsheet files. Choose the file specific to your lab section.*
2. This lab exercise will require student lab partners to have physical contact with one another. In certain religions and cultures, it is taboo for individuals to have physical contact with members of the opposite sex. We will be sensitive to needs of these students.

A brief background on statistical analysis

Why do you use statistical methods?
In this experiment, you wish to know, for example, if the index finger has a lower threshold of sensory discrimination than the upper arm. To be really sure, you would need to perform two-point discrimination test on every human being on earth. This is obviously impossible. Therefore, the strategy is to collect data from a small number of subjects (students in your lab section) as a sample, and make a general conclusion about the entire population. Statistical calculations will allow you to do this.

What can statistical methods tell you?
In this laboratory, we will collect data (threshold values for sensory discrimination) from all the students in the lab for each of the four parts of the body, and compute the means (same as the average) and standard deviation (a measure of variability). We will also perform "statistical hypothesis testing". Statistical hypothesis testing helps you to decide whether the observed difference (e.g., difference between index finger and upper arm) is likely caused by chance. We always ask this question: if there is no difference between the two (between index finger and upper arm) in the entire population, what is the probability of randomly selecting a sample (your lab section) with a difference as larger as or larger than what you observe in the sample? The answer is called the p-value. If the p-value is small, you conclude that the difference is statistically significant and unlikely to be due to chance. In the entire week, there will be seven lab sections, so we will have seven samples. If the p-value in your section is 0.03, this means that: "if there is really no difference between index finger and upper arm in the entire population, the chance for a given sample (any lab section) to observe the same or a larger difference in observed values is 0.03 (three out of one hundred)". In other word, even when there is no difference between the index finger and the upper arm in the entire population, if 100 samples (100 lab sections) are analyzed, 3 of them will have the same or a larger difference in the values between the index finger and the upper arm.

How small should the p-value be so that you can conclude that there is a statistical significance? It is a bit arbitrary, but often times we use 0.05 as the cut-off (if p-value is smaller than 0.05, the difference in observed values can be considered statistically significant).

In this laboratory, you just need to enter your own value under each column, and the template automatically calculates the mean and standard deviation. It also calculates the p-value for you. In case you want to know what formula was used, refer to the sites below:

mean: https://support.google.com/docs/answer/3093615
standard deviation: https://support.google.com/docs/answer/3094054
p-value: https://support.google.com/docs/answer/6055837

For your reference, you can use the same formulas in Excel spreadsheet.

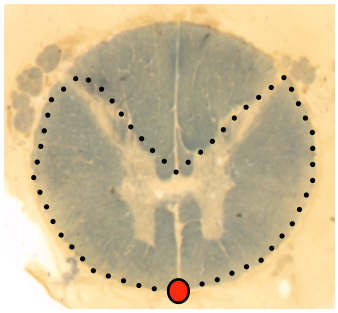
3. Answer the two clinical questions below in your lab report (0.75 point possible for each question; 1.5 points total)

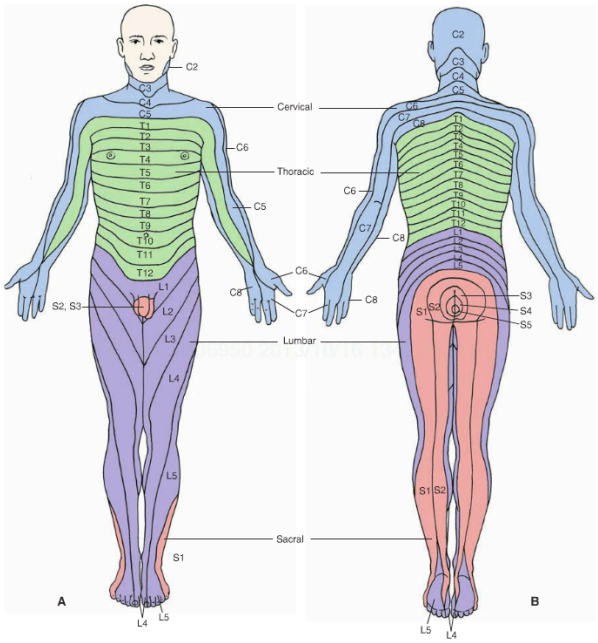
1) Below is a picture of the spinal cord at the lower thoracic level (T10). The anterior spinal artery (shown by a red dot) supplies blood to most of the spinal cord outlined by the dotted black line. When this artery is completely occluded only at this level of the spinal cord, what type of sensation will be affected? Choose from below and explain why (use anatomical descriptions of the affected region of the spinal cord) in two sentences. (answer: 0.3 point, reasoning: 0.45 point)

A. Vibration sense on your knee

B. Pain in your thigh

C. Pain on your chest



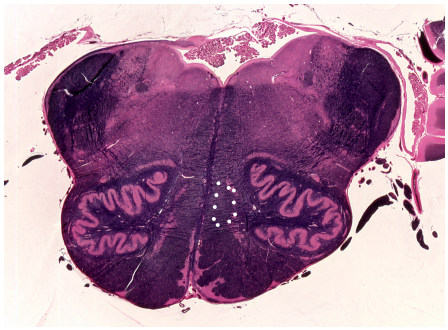


2) Your grandpa got a stroke that affected a limited region of his **right** medulla (outlined by a white dotted line). What type of sensation is expected to be lost? Choose from below and explain the answer with an anatomical reasoning in one sentence (answer: 0.3 point, reasoning: 0.45 point).

A. Pain in his left face

B. Pain in his right arm

C. Proprioception for his left arm



LAB 8 VISUAL AND AUDITORY SYSTEMS

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET

Report of LAB 8 is due at the beginning of your LAB 9.

Objectives:

1. Understand the visual and auditory pathways from the cranial nerves to the primary sensory cortex.
2. Identify structures of the visual and auditory pathways
3. Measure the visual field and find blind spots

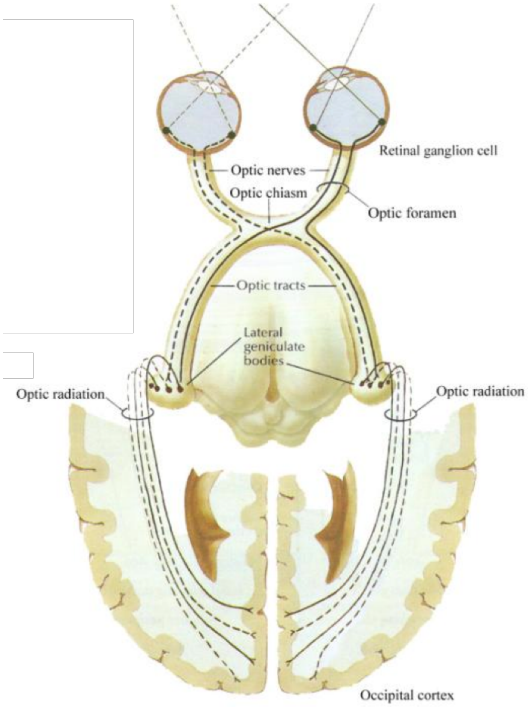
Materials:

1. Whole and half human brains
2. Dissected human brainstem
3. Histological slides of cross sections of the human brain
4. Tools for visual field test (vision disk, sight card and paper clip)

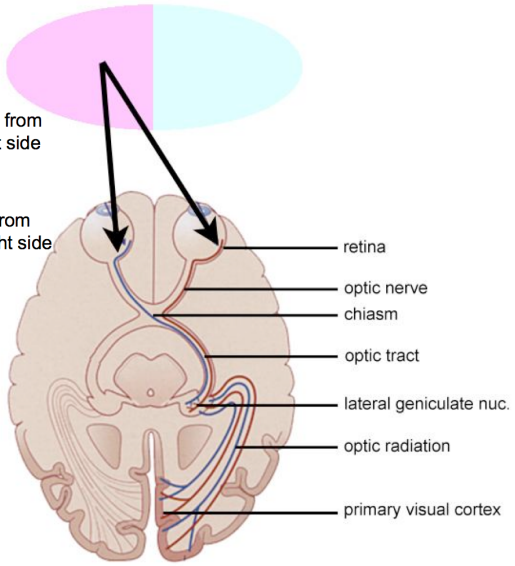
Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

1. Drawing of structures (1.6 points possible)
2. Report on visual field test (2.0 points possible)
3. Answer clinical questions (0.4 points possible)

Overview of central visual pathway:

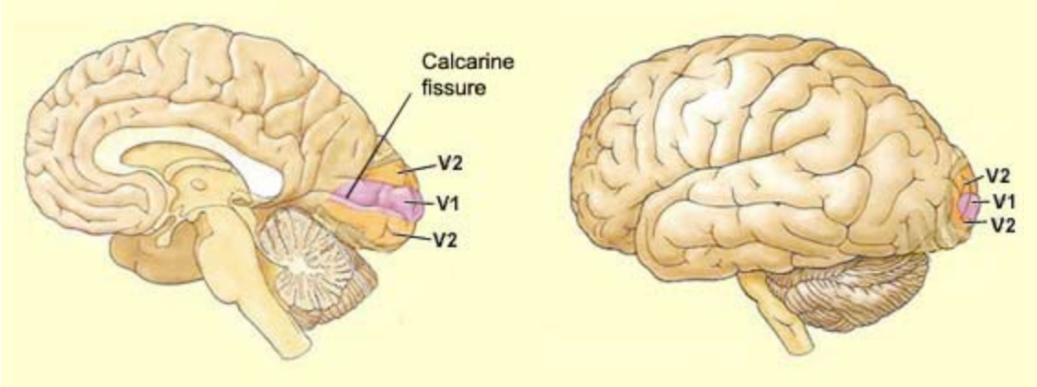


- The right visual hemifield from both eyes goes to the left side of the brain.
- The left visual hemifield from both eyes goes to the right side of the brain.

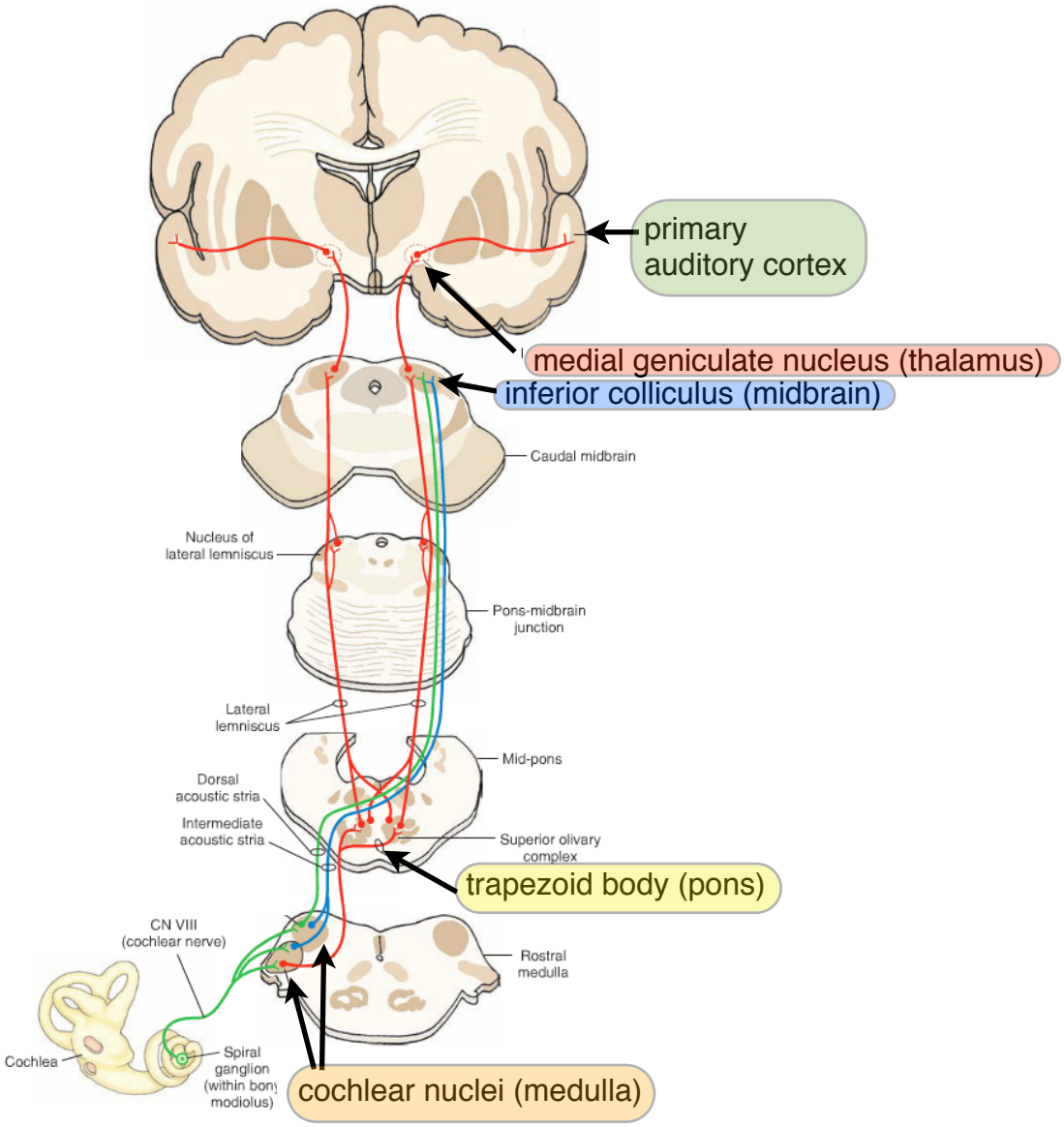


Central visual pathways from Dr. McLoon's lecture

Occipital lobe contains many visual areas. The primary visual area is the only one that receives direct innervation from the lateral geniculate nucleus of the thalamus. It is located both right above and below the calcarine sulcus (fissure) on mid-sagittal views, and on the posterior pole of the occipital lobe in lateral view (see below).



Overview of the central auditory pathway:

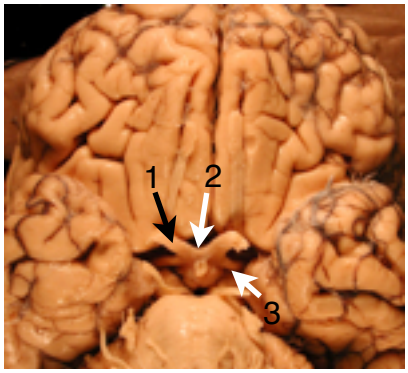


adapted from Siegel and Sapru
Fig.17-6

Procedure

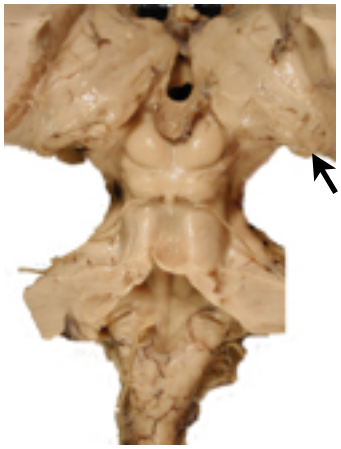
1. **Visual pathway on gross materials** (you may be asked to identify the labeled structures in exams)

A. Ventral view of the forebrain



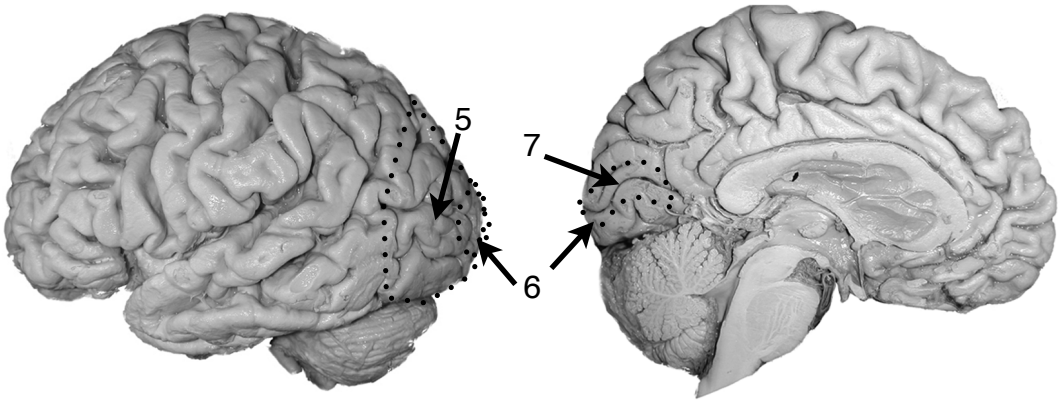
1.optic nerve (cranial nerve II)
2.optic chiasm
3.optic tract

B. Dorsal view of the thalamus and the brainstem.



4. lateral geniculate nucleus (of the thalamus)

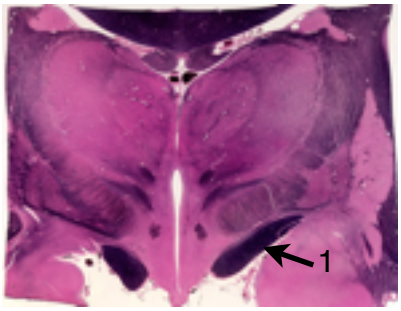
C. Lateral and medial views of the whole brain



5. occipital lobe (this includes the primary visual area)
6. primary visual cortex (V1)
7. calcarine sulcus (V1 is on both upper and lower “banks” of this sulcus)

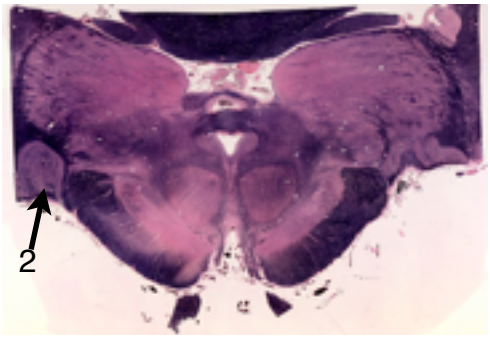
2. **Visual pathway on histological slides (going from rostral to caudal)**

(you may be asked to identify the labeled structures in exams)



1.optic tract

Slide 20 (diencephalon)

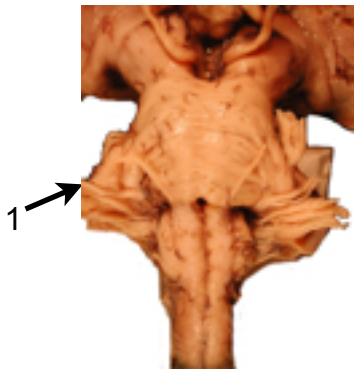


2.lateral geniculate nucleus

Slide 19 (diencephalon and midbrain)

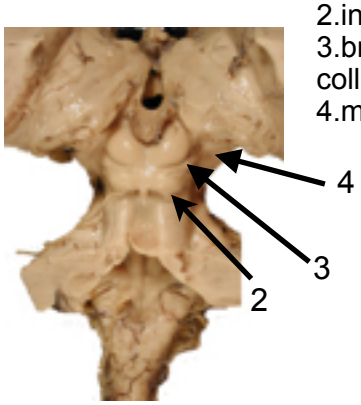
3. Auditory pathway on gross materials
(you may be asked to identify the labeled structures in exams)

A. Ventral view of the brainstem



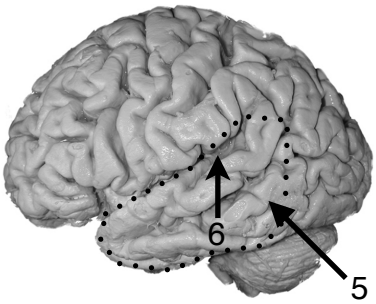
1. vestibulocochlear nerve (cranial nerve VIII)

B. Dorsal side of the brainstem



2.inferior colliculus
3.brachium of the inferior colliculus...a tract of axons from the inferior colliculus to the medial geniculate nucleus of the thalamus
4.medial geniculate nucleus (thalamus)

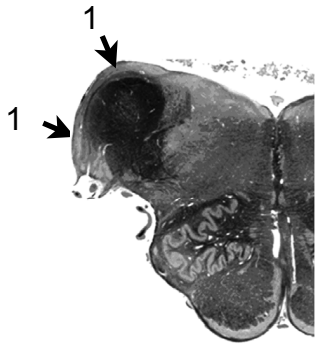
C.Lateral view of the whole brain



5. temporal lobe
6. primary auditory cortex (included in the temporal lobe)

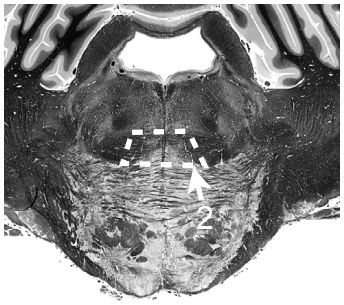
4. Auditory pathway on histological slides

(you may be asked to identify the labeled structures in exams)



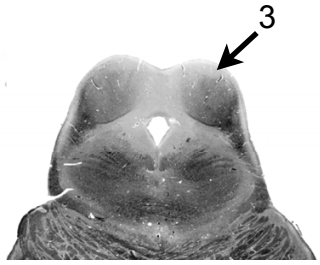
slide 9 (pons-medulla border on the dorsal side)

1.cochlear nuclei



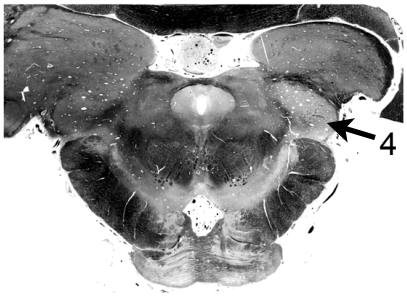
slide 12 (medulla)

2.trapezoid body..crossing fibers of ascending axons from the cochlear nuclei



slide 15-16 (midbrain)

3. inferior colliculus



slide 18 (midbrain)

4. medial geniculate nucleus (thalamus)

5. Draw the following structures in your lab report (0.4 point for each: 1.6 points possible)

1. the cochlear nuclei (in cross section of medulla)
2. the inferior colliculus (gross brainstem)
3. primary visual cortex (mid-sagitally cut human brain)
4. the lateral geniculate (cross section)

6. Test of the visual field (2.0 points possible for the lab report)

Tools
vision disk (one per group of 3 students)
sight cards
paper clips

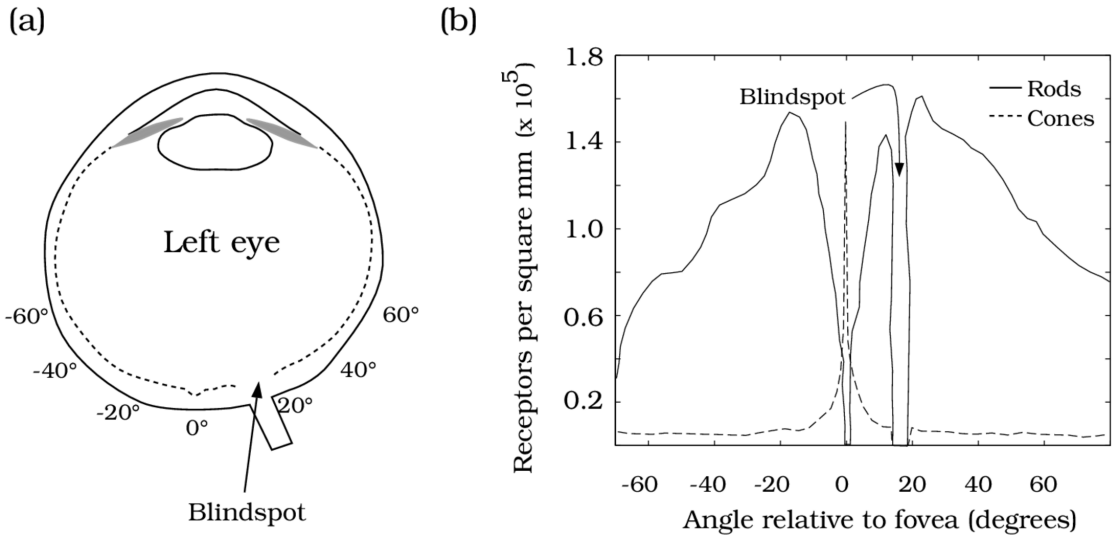
Background (taken from Finn Scientific Inc. http://www.flinnsci.com/media/452814/bf10896.pdf)
The retina, a thin tissue lining the back of the eye, contains specialized nerve cells, called photoreceptors, that are sensitive to light. The two different photoreceptors are known as rods and cones, so named because of their shapes.

Rod photoreceptors are:
-more numerous (over 120 million) than cones
-more concentrated around the periphery of the retina (see the figure below)
-more sensitive to light than cones and help us see in dim light.
-not sensitive to color (this is why it is difficult to distinguish colors in a dark room)
-very good at detecting motion (a moving object can usually be detected in the peripheral vision before the object can be clearly identified)

Cone photoreceptors are:
-found throughout the retina but the center of the retina (the macula) has a much higher density of cones than the periphery (the center of the macula, the fovea, has the highest density of cones and has the highest visual acuity)
-responsible for color vision (even though the eye has fewer cone receptors (6–7 million) than rod receptors, cones are vital to our central vision and the ability to see fine details)
-used primarily when we read

Try focusing on a letter in the middle of a word in a sentence and see how many other words you can read to the right or left without moving your eyes. When one looks at something directly, the image is focused on the macula. When something is seen out of the corner of the eye, the image is focused on the periphery of the retina, where more rods and fewer cones are found.

The blind spot
-corresponds to the optic disk, where the ganglion cell axons exit the retina
-there are no photoreceptors in the blind spot



The distribution of rod and cone photoreceptors across the human retina

Procedure

1. For Vision Disk experiments, choose one member of the group to be the subject, and one to be the tester. The third person will watch the eyes of the subject so they do not move (no saccades allowed!) during the experiment. After the experiment is completed, rotate the roles.

2. Use the supplied instruction manual and measure the following:
    a) peripheral visual field
    b) reading visual field (read the letters on the card)
    c) color visual field (identify the color of the dot on the card)

-Measure the extent of the visual field at least three times in each person and take the average of these values.
-the sum of the right and left visual field represents the range of your peripheral visual field or reading visual field and color visual field. Record the ranges in the Google spreadsheet on the provided iPad.

3. Use the Vision Disk, find the position of the blind spot. Use a color dot on the card, do the same experiment as above. The tester will slowly move the card in the right visual field and the subject will find the angle where the he/she cannot see the color dot without moving the eyes. Do this three times for each subject and take the average of these values. Enter the value in Google spreadsheet.

your subject #=	**your own measurements (degree)**	**mean value for the whole section (degree)**	**standard deviation (degree)**
Test	*(this column=0.8 point)*	*(these two columns=0.4 point)*	
extent of peripheral visual field			
extent of reading visual field			
extent of color visual field			
position of the blind spot			

You should now fill the table below **(0.8 point for your own measurements, 0.4 point for average and standard deviation of the entire group; <u>1.2 points possible for this table</u>)**

Discussion (write in your lab report: 0.8 point possible)

1. **Find out the p-value for the comparison between peripheral visual field and reading visual field (0.2 point). Why is the reading visual filed much narrower than the peripheral visual field? Explain in one sentence (0.3 point)**

2. **Why do you think is the peripheral vision important? In what situation in your life do you use peripheral vision? Explain in one sentence (0.3 point)**

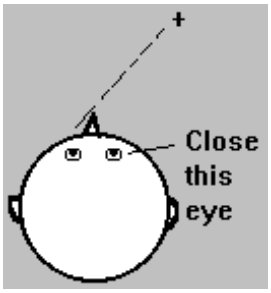
-Research has shown that cell phone usage in an automobile severely limits use of peripheral vision, even for a period of time after the driver has stopped using the phone. Discuss the need for peripheral vision while driving, and debate the pros and cons of restricting cell phone usage in cars.

-Some animals have wide peripheral vision to better detect predators. The wider field of view provided by side-facing eyes and monocular vision provides better adaptation, reducing the chance that a predator could approach them unaware. In many hunting animals, binocular vision is common, because it is more important for them to accurately determine the distance between themselves and the prey.

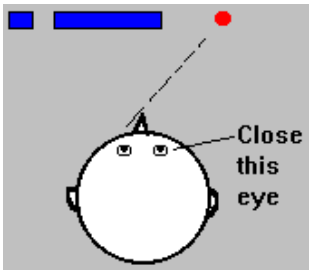
(optional activity)

Simple tests of your blind spots and adaptation

The blind spot is the area on the retina without receptors that respond to light. Therefore an image that falls on this region will NOT be seen. It is in this region that the optic nerve exits the eye on its way to the brain. To find your blind spot, close your right eye. Hold the image (or place about 20 inches away. With your left eye, look at the +. Slowly bring the image (or move your head) closer while looking at the +. At a certain distance, the dot will disappear from sight...this is when the dot falls on the blind spot of your retina. Reverse the process. Close your left eye and look at the dot with your right eye. Move the image slowly closer to you and the + should disappear.



Here is another example that will help you find your blind spot. For this image, close your right eye. With your left eye, look at the red circle. Slowly move your head closer to the image. **At a certain distance, the blue line will not look broken!! This is because your brain is "filling in" the missing information.**



7. Answer the clinical question below in your lab report (0.4 point possible)

Which part of the visual field is affected with a stroke that damages most of the left occipital cortex? Choose one answer and explain why in one sentence (answer: 0.2 point, reasoning: 0.2 point).

A. right visual field for both eyes
B. left visual field for both eyes
C. left and right visual field for the right eye

LAB 9: MOTOR SYSTEM

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
TURN IN YOUR REPORTS FOR LAB 8

Report of LAB 9 is due at the beginning of your LAB 10.

Objectives:
1. Identify and name the structures involved in the production of movement
2. Follow each of the motor pathways from the motor cortex to lower motor neurons
3. Perform experiments on sensory-guided motor response.

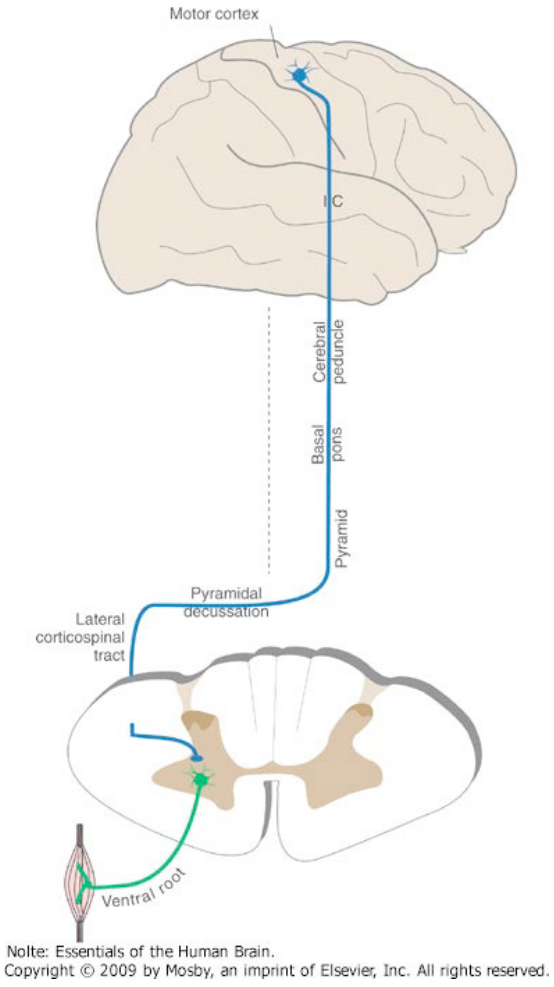
Materials:
1. whole and half human brains
2. dissected human brainstem
3. human spinal cord
4. histological slides of cross sections of the human brain and spinal cord
5. reflex hammer and EMG Spikerbox for testing stretch reflex (one per group)
4. EMG Reaction Timer for measuring reaction time (one per group)
8. iPad (provided; one per group)

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

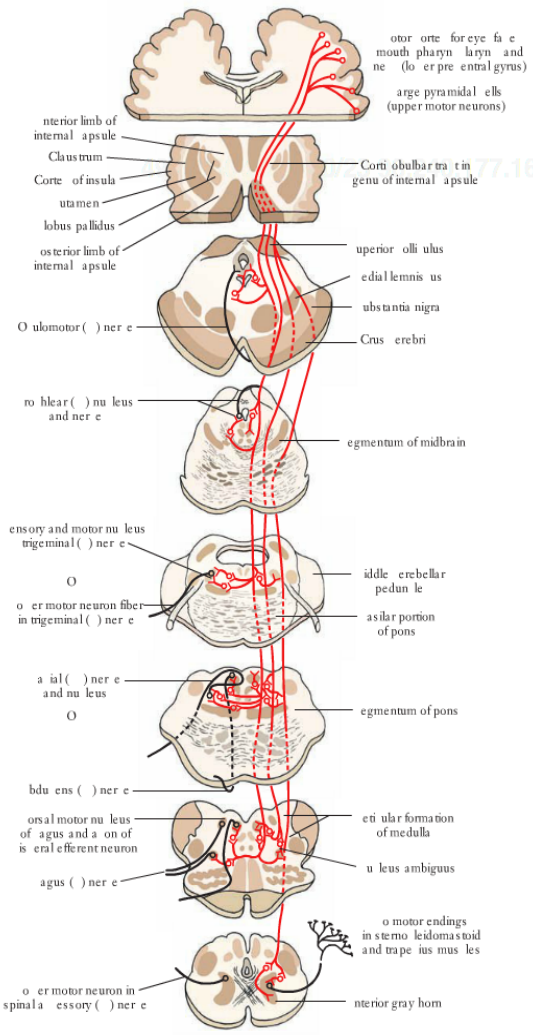
1. Drawing of structures (1.2 points possible)
2. Report on response time experiments (2.0 points possible)
3. Answer clinical questions (0.8 points possible)

Overview of the motor pathways:

corticospinal tract



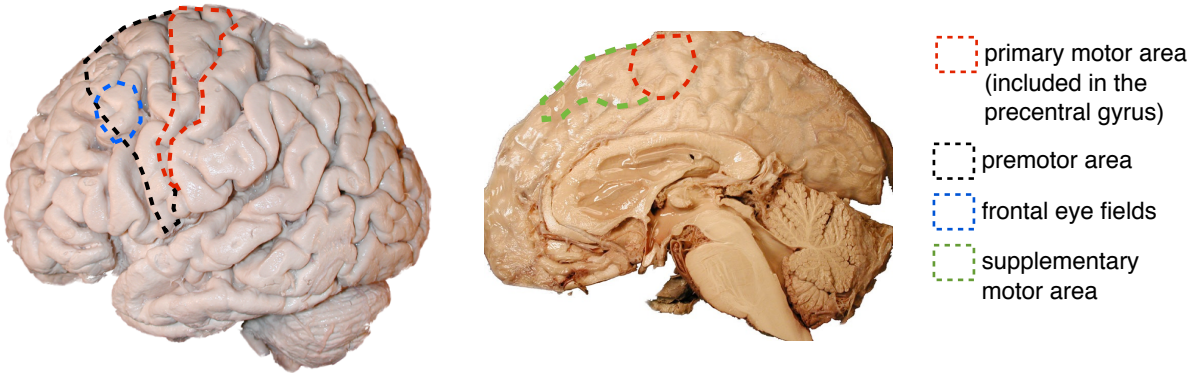
corticobulbar tract



Procedure
1. Motor pathway on gross materials

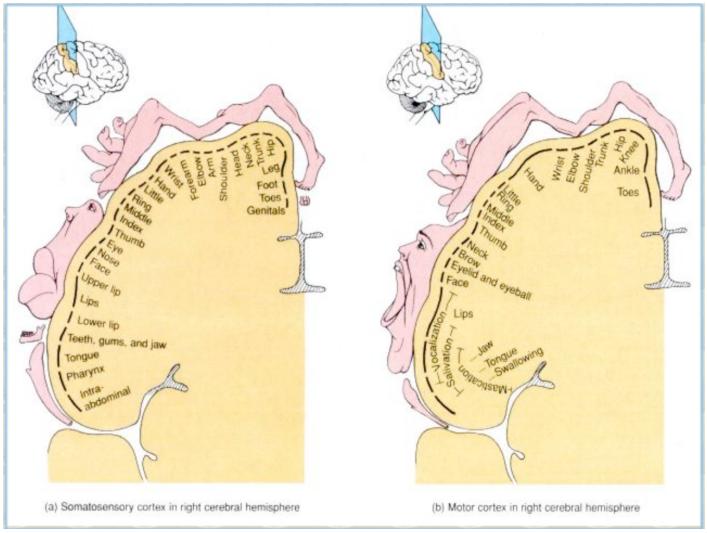
A. Identify the following on whole and half human brains
-precentral gyrus including primary motor cortex
-premotor cortex
-frontal eye field
-supplementary motor cortex

Remember that all of the above areas contain neurons that project axons down to the spinal cord. The premotor cortex, frontal eye field and supplementary motor cortex project axons to the primary motor area.



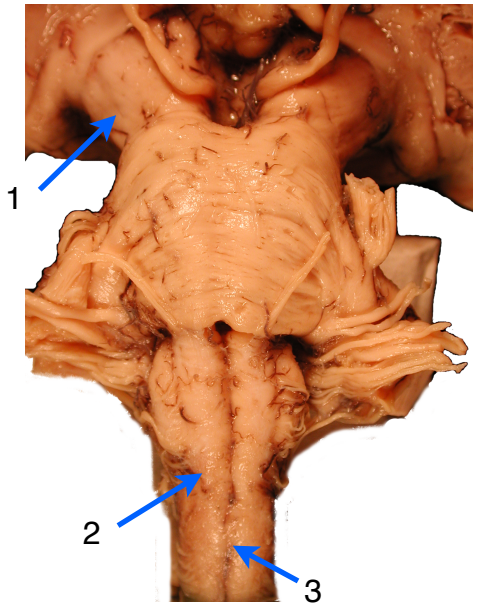
Primary motor area is topographically organized, just like the primary somatosensory area (see homunculus diagram below).

medial/dorsal part: leg, genitalia
lateral/ventral part: face, tongue



sensory homunculus motor homunculus

B. Identify the following on dissected human brainstem

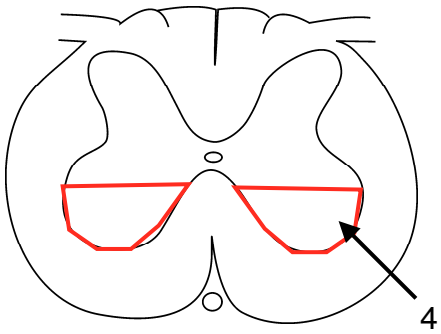


1. cerebral peduncle (midbrain)
2. pyramid (medulla)
3. pyramidal decussation (medulla-spinal cord junction)

Corticospinal tract runs through various structures (and changes its name) as it descends from the cortex to the spinal cord.

-internal capsule, posterior limb (forebrain)
-cerebral peduncle (midbrain)
-pyramidal fascicle (pons)
-pyramid (medulla)
-pyramidal decussation
-lateral funiculus (spinal cord)

C. Human spinal cord



ventral horn (spinal cord)..includes motor neurons

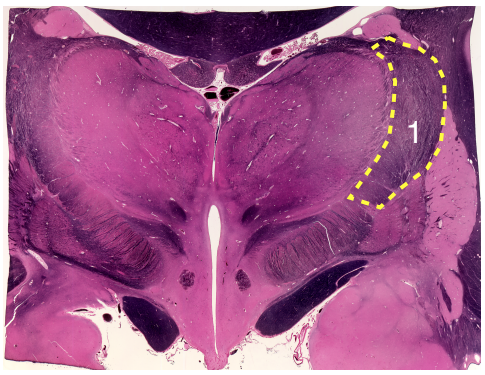
Also identify the ventral roots on gross spinal cord.

2. Motor pathway on histological slides of cross sections of human brain and spinal cord

The corticobulbar tract is composed of the axonal fibers of cranial nerve motor neurons (in the motor cortex). "Bulb" is an archaic term for the medulla (or medulla + pons).

These axons accompany the corticospinal tract until they reach the level of the nuclei in which they terminate.

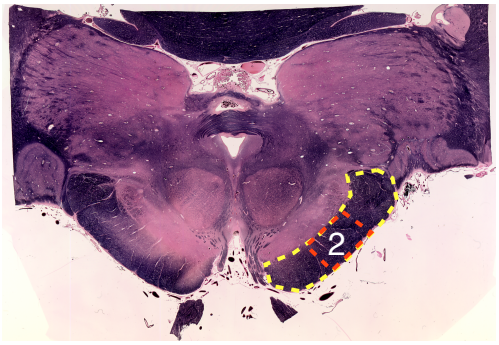
Unlike the corticospinal tract, the corticobulbar tracts project bilaterally (with some exception; see the schematic in page 66).



Slide 21-22

internal capsule, posterior limb (forebrain)

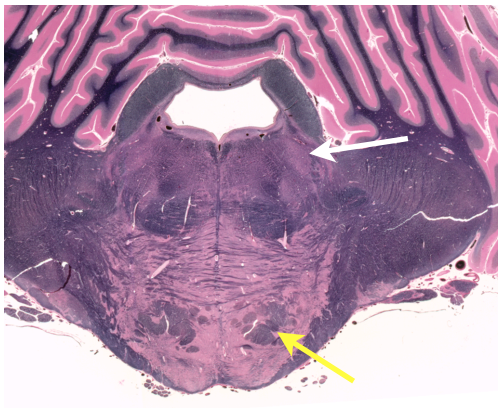
Internal capsule is shared by many other axon bundles including sensory axon bundles from the thalamus to the cortex.



Slide 18

cerebral peduncle (midbrain)

Note that only the middle third of the cerebral peduncle (red) includes the corticospinal tract.



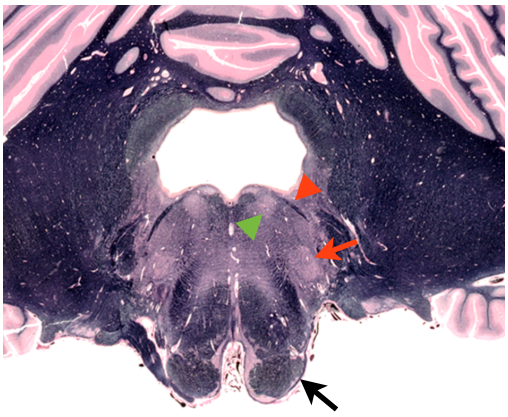
Slide 12

pyramidal fascicle (pons)...yellow arrow

The pons contains two types of fascicles: 1) pontine fascicle, which are the axons of pontine neurons that run medial to lateral, decussating to the contralateral side, arriving at the cerebellum via the middle cerebellar peduncle, and 2) the pyramidal fascicles, which are axons of the corticospinal and corticobulbar tracts that run rostral to caudal.

trigeminal motor nucleus (pons)...white arrow

Axons of neurons in the trigeminal motor nucleus innervate jaw muscles.

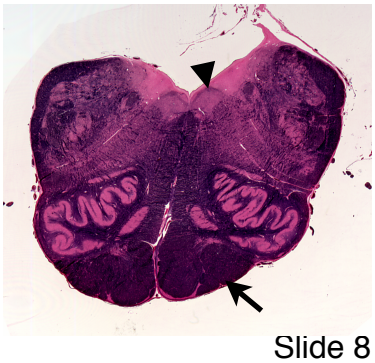


Slide 11

facial nucleus (pons)...red arrow

Axons of neurons in the facial nucleus innervate muscles of the face. The axons from this nucleus (red arrowhead) loop around the abducens nucleus (green arrowhead) and leave the brainstem at the ventral surface.

pyramid...black arrow (also observed in the gross material)

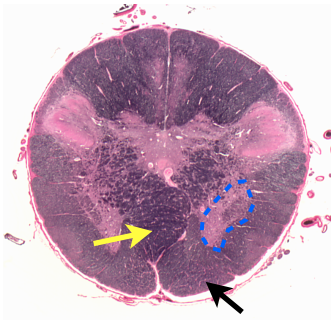


Slide 8

hypoglossal nucleus (medulla)...arrowhead

Axons of neurons in the hypoglossal nucleus innervate the tongue muscles.

pyramid...arrow



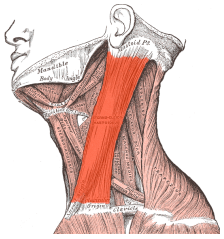
Slide 3

Slide 3 shows a section of a cross section of lower medulla very close to the spinal cord

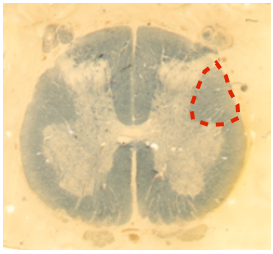
spinal accessory nucleus...blue dots

Axons of neurons in the spinal accessory nucleus innervate muscles on the shoulder and neck; trapezius and sternocleidomastoid.

pyramid (black arrow)
pyramidal decussation (yellow arrow)
(both structures were observed in the gross material)



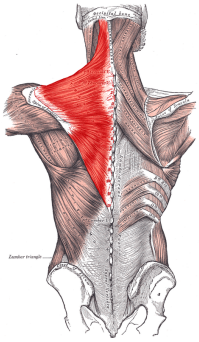
sternocleidomastoid muscle



Slide 54

lateral funiculus (dorsal part) in the spinal cord...red dots

In the spinal cord (after the pyramidal decussation), the corticospinal tract runs in the dorsal part of the lateral funiculus.



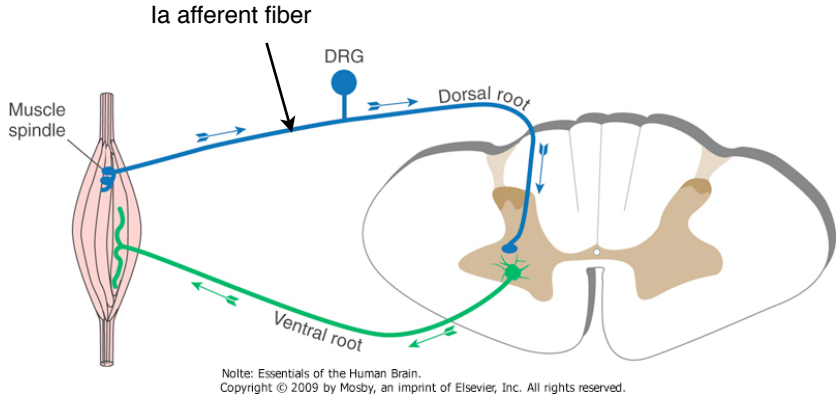
trapezius muscle

3. In your Lab Report, draw the outline of the following slides (not shown in the lab manual) and label the corticospinal tract with the correct name (0.3 point for each: 1.2 points possible)

1) slide 19 or 20 (cerebral peduncle in the midbrain)
2) slide 15 or 16 (pyramidal fascicle in the pons)
3) slide 9 or 10 (pyramid in the medulla)
4) slide 5 (pyramid in the medulla)

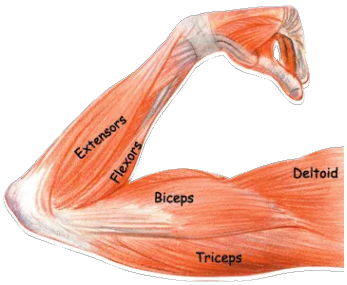
4. Activities to understand the integration of sensory and motor pathways (2.0 points possible for the lab report)

1) Practice in eliciting muscle stretch reflex



This is a test of monosynaptic reflex involving the sensory neurons and motor neurons.

You can elicit this type of reflex in many parts of your body. These tests are clinically used to evaluate the location of lesions. For example, lesions of descending motor system **above** the lower motor neurons (either in the ventral horn of the spinal cord or cranial motor nuclei) will generate exaggerated reflex (hyper-reflexia). Lesions in the motor neurons or their axons will cause reduced reflex (hypo-reflexia). Examples of reflexes that are clinically useful are:



1) upper extremities
biceps reflex (tap the biceps muscle)
triceps reflex (tap the triceps muscle)
http://www.youtube.com/watch?v=2sm4ynlzEi8

2) lower extremities
knee jerk (tap the patella)
ankle jerk (tap the Achilles tendon)
http://www.youtube.com/watch?v=3PILgkVKIAg

Take turns with your group members testing each other's reflex. Among the reflexes above, the ones in the lower extremities will be easier to elicit, but you can try other ones if you wish. You can test:

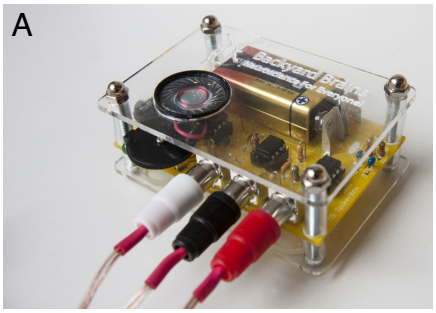
For the knee jerk (patellar reflex), the latency will be about 30ms. The lack of a reflex may indicate a lesion that involves one or more of the components of the reflex arc. Some healthy people show weak or no reflex. You can increase a reflex by the following maneuver:

Jendrassik maneuver: the person forcefully contracts a muscle of the forearm or the jaws while the examiner tries to evoke a reflex in the lower extremities. This may be due to the descending (corticospinal) signals that increase the excitability of the reflex center.

2) Electrophysiology of muscle contraction using an EMG Spikerbox

We will then “hear” and “see” muscle contractions using a electrophysiological device (electromyogram; EMG). Each group of students will be provided with a recording box (EMG SpikerBox; Fig.A) (from Backyard Brains). The box contains an amplifier and a speaker to make the electrical activity audible. An output port is available for laptop, tablet or smartphone recording. We provide an iPad for each group so you can visualize the muscle action potentials.

A



B

smartphone cable

C

EMG electrode cables

A. Recording from your biceps muscles

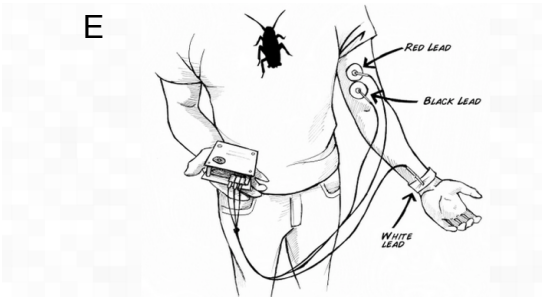
[Procedure] watch the video: https://backyardbrains.com/experiments/muscleAP

1. Connect three EMG electrode cables to the box (make sure the color matches; Fig,A, C)
2. Connect the EMG SpikerBox to the iPad using a green “smartphone cable” (Fig.B). **Be careful: each end of the cable needs to be connected to the right device**
    a. iPad is connected to the end labeled as “Smartphone”.
    b. EMG SpikerBox is connected to the end labeled as “SpikerBox”. There are two cable connectors on the shorter side of the EMG SpikerBox. Use the one that is closer to the battery.
3. Connect the other end of each EMG electrode cable to:
    a. white (ground): your forearm (use an EMG electrode pad to attach it). You can clip the end of the cable to your ring if you have one.
    b. black and red: EMG electrode pads (Fig.D) on your biceps muscle (Fig.E). Use the arm that does not have the ground electrode.

D

4. Launch Spike Recorder App from the iPad. Pinch the screen so that you can change the scale on the x-axis (time) or y-axis (amplitude)
5. Turn on the EMG SpikerBox (rotate the black dial **all the way**).
6. Make sure that the background noise is minimum without movement of your arm. A nearby electronic device connected to an AC adaptor will cause a noisy background activity.

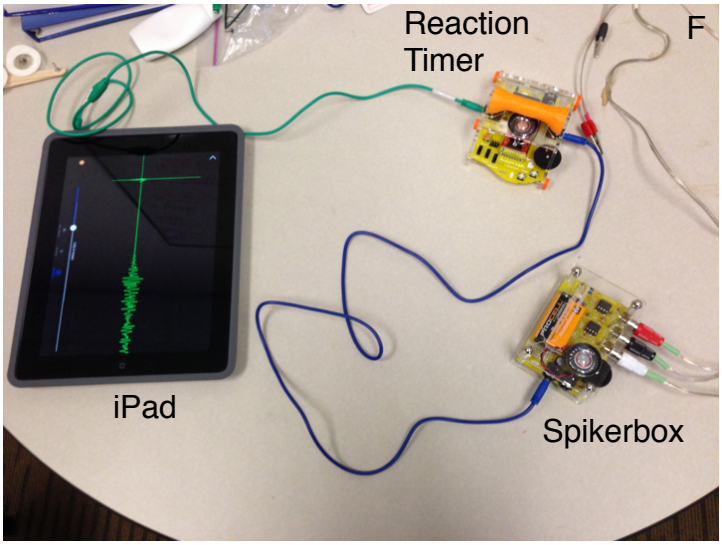
E



7. Contract your biceps and find the muscle action potentials on the iPad.
8. Pinch the iPad screen both horizontally and vertically to change the gains (time for the horizontal axis and the amplitude for the vertical axis). Find the optimal gains to see the muscle activity.

[Caution] Batteries die very quickly in SpikerBox. If you do not see a response, ask instructors to change batteries and see if it fixes the problem.

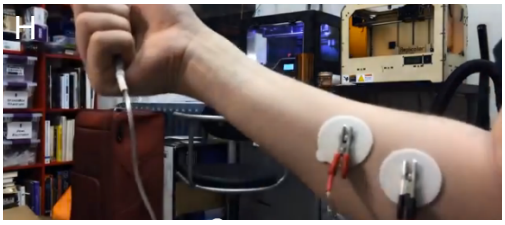
3) Measurement of response time using an EMG SpikerBox and an EMG Reaction Timer



In this experiment, you will test sensory guided motor control by using a combination of two devices, EMG Reaction Timer (for generating visual and/or auditory stimuli) and EMG SpikerBox (for recording). You will measure the time it takes to respond to visual or auditory stimuli and move your muscles. You will determine if the reaction time differs between different stimuli.

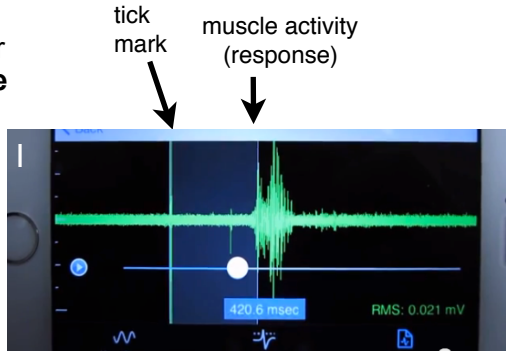
Each group of three students will have one complete set of devices. Take turns within the group so that everyone will have her/his reaction times measured. Each experiment needs one subject (who responds to stimuli) and at least one tester (who generates stimuli using the EMG Reaction Timer).

[Procedure] watch the video: https://backyardbrains.com/experiments/EMGReactionTimer



1. Connect EMG SpikerBox and EMG Reaction Timer using a blue cable.
1. Connect EMG Reaction Timer to the iPad using a green cable. Now the three devices are connected in tandem (SpikerBox→Reaction Timer→iPad) (Fig.F).
2. Turn on Reaction Timer and SpikerBox (all the way up).
3. Choose the light stimulus (the left-most switch in Fig.G should be up; the other two black switches (tone, random) should be down so they are turned off for now.
4. There are two stimulus boutons on the right side (Fig.G). Push the left one to provide the light stimulus.
5. The subject is now connected to the SpikerBox. Try forearm muscles first using EMG electrode pads (Fig.H).
6. The subject will flex the muscle as quickly as possible in response to the light stimulus. You should see a tick mark at the stimulus and EMG signals as you contract your muscles (Fig.I). If necessary, zoom in and out (both horizontally and vertically) using your fingers on the iPad.
7. After a few tests, begin to record the data by pressing the red round button at the upper right corner of the App window of iPad. After at least 20 stimuli/responses, tap the top, red part of the App window to stop the recording.

8. On the App, tap “Recording” to show a list of your recordings. You can rename each recording for your convenience. Tap the “Play” button to **play back the recording**. Pause the playback when you find a tick mark and a robust EMG signal. Place your fingertip on the tick mark for a second or two until you see a white vertical line; then swipe from there to the first noticeable EMG signal to measure the reaction time (Fig.I). **Measure the reaction time** for 10 responses and record the results in the table in the next page.



9. Turn on the “tone” function of Reaction Timer (Fig.G) and turn off the “light” function. To avoid the noise that continues from the tick mark, minimize the power of the tone stimulus (just generating a clicking sound, not a loud tone, should be enough for the test). Repeat the experiment (steps 7-8) in response to tones. Use the left white bouton to generate a tone stimulus. **Measure the reaction time** for 10 responses and record the results in the table in the next page.

10. Calculate the mean reaction time for each stimulus and enter the values in the table as well as the Google Sheet on your iPad.

11. Delete all the recording from the iPad at the end of the lab. **You can touch “Share” button to email the files to yourself for later analysis just in case.**

Table 1 *(this table=0.5 point)*	**your own measurements (msec)**	
Reaction time (msec)	**Audio**	**Visual**
Trial 1		
Trial 2		
Trial 3		
Trial 4		
Trial 5		
Trial 6		
Trial 7		
Trial 8		
Trial 9		
Trial 10		
Average Reaction time		

Table 2 **Average reaction time (msec)** *(this table=0.3 point)*	**your own measurements (msec)**	**mean value for the whole section (msec)**	**standard deviation for the whole section (msec)**
Visual			
Audio			

In the lab report, include the following discussion in addition to filling the two tables above.

a. **Find out the p-value for the comparison between visual and tone stimuli (0.2 point).**

b. **Is there significant difference in reaction time between tone and light stimuli? If so, explain why there is difference (in one sentence). (0.5 point)**

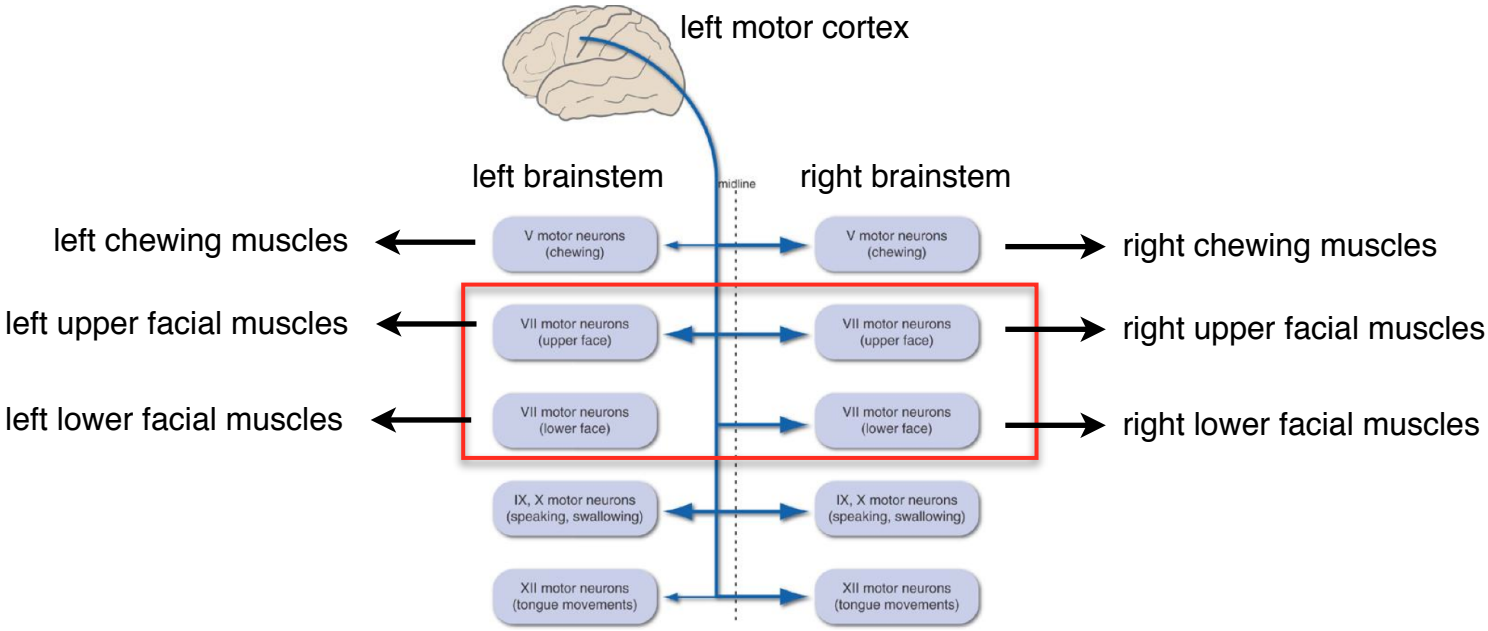
b. What explains the variation in measurements between individuals (in one or two sentences)? (0.5 point)

4. Answer the two clinical questions below in your lab report (0.8 points total)

As you saw in Dr. McLoon's lecture (see the diagram to the left below), the lower face and the upper face have a difference in the laterality of innervation from the cortex. The facial nucleus innervating the **lower** face is largely controlled by <u>contralateral</u> motor cortex, whereas the facial nucleus innervating the **upper** face is <u>bilaterally</u> controlled by the motor cortex. Axons of motor neurons in the facial nucleus project to the ipsilateral muscles without crossing at the midline. How are the motor functions of the facial muscles affected in the following situations? Justify each of your answers in one sentence.

a) a lesion in the left internal capsule involving fibers going to the facial nucleus (**0.4 point possible)**

b) a left facial nerve lesion ("Bell's palsy") (**0.4 point possible)**



LAB 10: ELECTROPHYSIOLOGY OF MECHANOSENSATION

FIRST, SIGN IN ON THE LAB ATTENDANCE SHEET
TURN IN YOUR REPORT FOR LAB 9

Report of LAB 10 is due on December 3. Drop the lab report at Department of Neuroscience Office (6-145 Jackson Hall). There will be a designated folder at the front desk.

Part of this lab manual was adapted from:

Oakley and Schafer (1978) Experimental Neurobiology: A laboratory Manual. University of Michigan Press
French and Sanders (1981) The mechanosensory apparatus of the femoral tactile spine of the cockroach, Periplaneta americana. Cell Tissue Res 219: 53-68

Backyard Brain website:
https://backyardbrains.com/experiments/

Objectives:
1. With the cockroach leg preparation, hear and see (using the iPad Spike Recorder app) the dramatic changes in activity caused by touching the mechanosensitive barbs.
2. Understand the concept of voltage when recording action potentials propagating in the nerve
3. Test sensory adaptation

Materials:
1. SpikerBox (one per group).
2. iPad with the Spike Recorder software (one per group)
3. Cockroaches (approximately three per two groups). We will use their legs for electrophysiological recording. One leg is likely to last for the entire session, but if the activity becomes difficult to detect, you should get a new leg. It is ideal to change legs after each manipulation.
4. toothpicks and straws (for stimulation)
5. magnifier
6. forceps and pins (to move the leg)
7. counter (one per group)

Lab Report (5.0 points possible for this lab) *attendance point: 1.0, lab report point: 4.0*)

Table 1 (1.4 point)
Table 2 (1.4 point)
Table 3 (1.2 point)

Background 1: cockroach legs

Detection of touch, vibration and airborne sound are all examples of *mechanoreception* of external stimuli. Detection of changes in muscle length or tension as well as joint position is a type of internal mechanoreception, which has been termed *proprioception.* The ability to detect external mechanical stimuli is of obvious value to the animal, but proprioception can be considered to be equally important because it is vital to central nervous control of postural adjustments and movements. One of the simplest biological preparations for the study of both touch and joint receptors is the insect leg. In this lab, we will use cockroach legs to record sensory responses using an electrophysiological device and an iPad.

Cockroach legs have a number of cuticular spines (Fig.1A) that function as sense organs. Most of the spines are on the tibia (segments of the leg are coxa, femur, tibia and tarsus, in a proximal-to-distal order). The femur has only one spine that points outwards near the border with the tibia. Each spine is mounted in a flexible socket and is innervated by a single sensory neuron which conducts information to the central nervous system (Fig.2,3). External movement of a spine produces action potentials that propagate in the afferent axons. These axons form the nerve within the leg. By placing electrodes near these axons, we can record these action potentials.

In addition to the cuticular spines, insect legs have other sensory organs. One of them is called the campaniform organ, which is usually found near joints (Fig.1B). Joint movement will elicit the response of this organ.

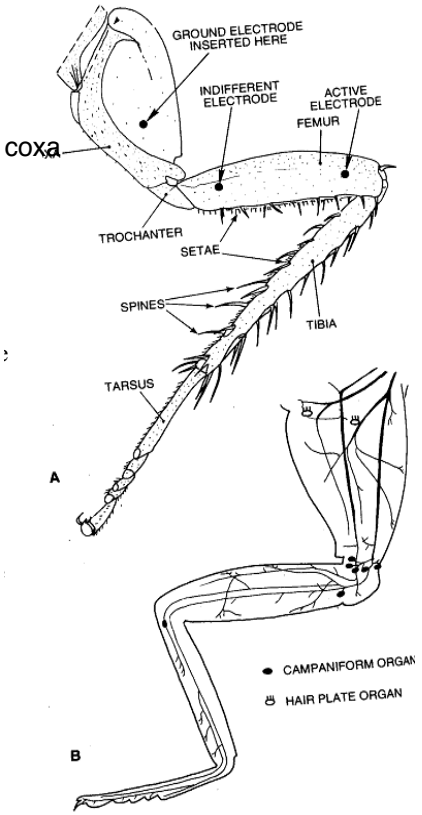


Fig.1. **A.** Anterior view of a cockroach leg showing the locations of cuticular spines. Sites of electrode placement are also shown. **B**. Innervation of the cockroach leg and the sites of campaniform organs associated with joints.

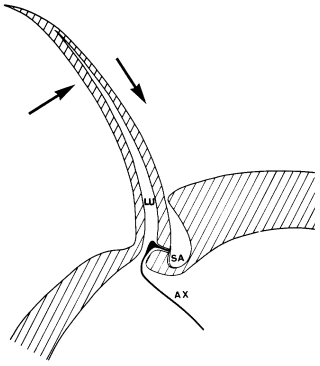


Fig. 2. A schematic representation of a tactile spine and the associated sensory neuron (SA) and its axon (AX). The arrows indicate the directions of the movement of the spine that elicit a strong response from the sensory neuron.

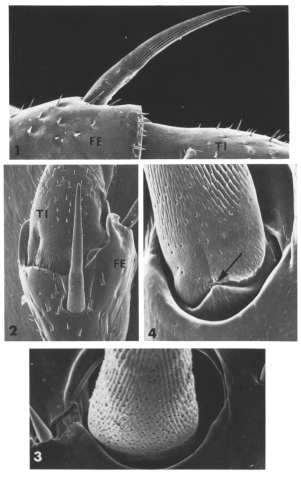


Fig. 3. An electron micrographic view of a tactile spine and the socket

Background 2: action potentials

When a neuron is stimulated and the membrane potential becomes less negative (depolarization), an action potential is generated due to the rapid influx of Na^+ ions through the opening of voltage-sensitive sodium channels. This is followed by the efflux of K^+ ions, which will result in hyperpolarization. Action potentials propagate down the axonal membrane and convey information to the post-synaptic cell.

There are two different ways to measure the action potentials by electrophysiology. **Intracellular recording** is to place one electrode inside the cell and the other one (ground electrode) outside the cell. This method measures the voltage (charge) or current (movement of ions) across the cell membrane. With the SpikerBox, we will use the other method, **extracellular recording.** With this method, both electrodes are placed outside of the cell (axons). Measurement of the voltage and current will appear inverted compared with intracellular recordings (remember what we saw in the simulation in LAB 4).

Another difference between intracellular recording and extracellular recording is that with intracellular recording, the amplitude of action potentials is consistent during an experiment, but with extracellular recording that is not the case.

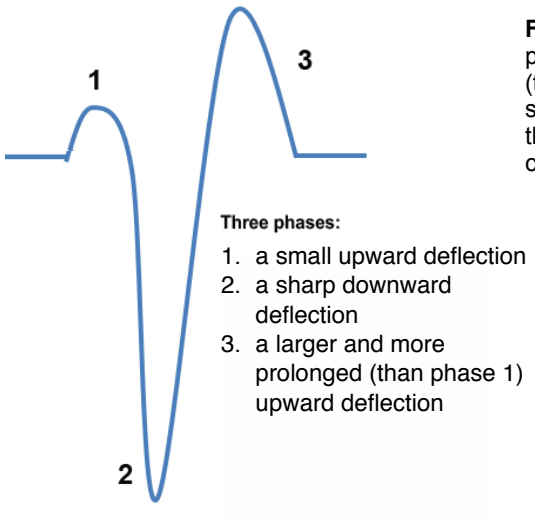


Fig. 4. A typical wave form of an action potential obtained by extracellular recording (this figure will help you to remember the shape of a typical spike. The generation of the spike shape actually depends on changes in currents)

Because voltage is a measure of the difference between two points, the SpikerBox electrode cable has two needles; we are measuring the voltage between the two electrode pins (black and white). There is another electrode (red) that is called the "ground" electrode". This will help canceling out the noise from the environment (e.g. 60Hz noise associated with the general-purpose alternating current (AC) power supply).

Action potentials that propagate along the nerve are small, and must thus be amplified by devices like your SpikerBox in order to be detected. Even without stimulating the leg, there is a spontaneous activity. We will observe this first. One measure of the spiking activity is to measure the frequency of spikes. Using the recording capability of the iPad App (Spike Recorder, the same one that you used in Lab 9), we will count the number of spikes within a certain duration of time and calculate the spike frequency.

In this lab, we will use cockroach legs and a set of devices (SpikerBox and iPad) to record the response of sensory neurons to various stimuli. We will then test the effects of adaptation to the activity of these neurons.

Procedure:

1. Examination of the cockroach leg

Look at the website before you come to the lab:

https://backyardbrains.com/experiments/spikerbox

There will be a “cockroach station” on one of the benches, where you can anesthetize a cockroach and remove their leg for your experiments. Place a cockroach in a container with ice water. Keep it there for a few minutes until the insect stops moving (Fig.5A).

Take out the cockroach and remove a meta- or meso-thoracic leg (Fig.5B) **by cutting the leg as closely as possible to the body** ensuring that you include as much of the coxa as you can. Meta- and meso-thoracic legs are large and easy to handle.

Return the insect to its house. Briefly examine the isolated cockroach leg under a magnifier. Examine the major parts of the leg, which are coxa, femur, tibia and tarsus (Fig.1A).

Fig. 5A

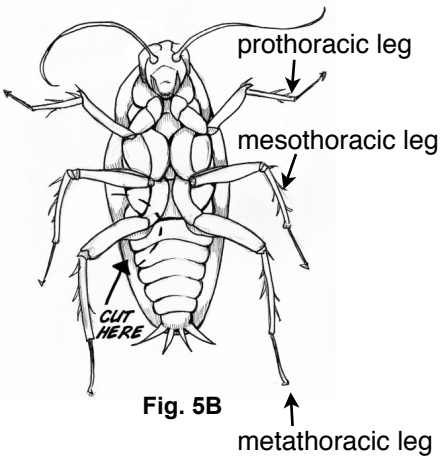


Fig. 5B

2. Start the electrophysiological recording

Look at the website: https://backyardbrains.com/experiments/spikerbox and watch the video.

A. Connect the red and white electrode cables to the SpikerBox (Fig.6). Do not connect the SpikeBox to the iPad for now.

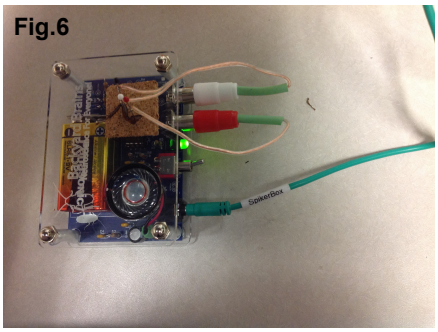


Fig.6

Red cable: has a ground electrode (purpose: to reduce the noise in the environment)

White cable: has two recording electrodes (black and white)

We will measure the difference of voltage between the black and the white electrodes.

B. Place the cockroach leg on the cork of your SpikerBox. Insert the red electrode into the coxa and the black and white electrodes into the femur (Fig.1A and Fig.7). Make sure that the three electrodes do not touch each other. Then, using forceps, lift the entire leg off the cork so that the leg is penetrated just by the three electrodes without touching the cork base (Fig.7). This will help further reducing the background noise when you record. It may also help to turn off your cell phone for a noise reduction.

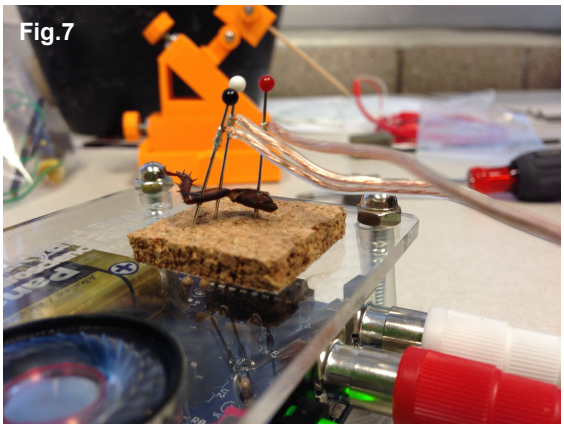
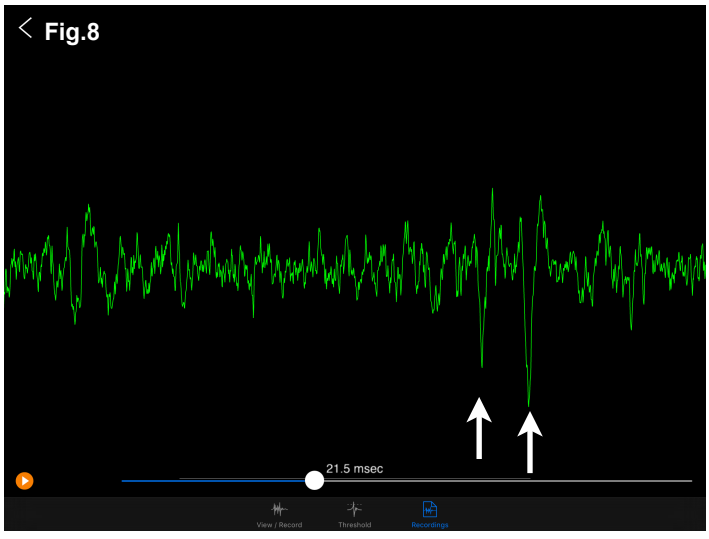


Fig.7

C. Turn on SpikerBox (the indicator light is green now). Do you hear a “popcorn” sound coming out of the SpikerBox? An example can be heard in the following link.

https://www.backyardbrains.com/experiments/files/recordings/CockroachSpikes.mp3

This is the spontaneous spiking activity of the nerve in the leg.



D.Connect SpikerBox to iPad using a green smartphone cable (you need to insert the plug into the iPad as far as it will go; otherwise, the sounds are picked up by the iPads microphone and displayed on the scren). **Be aware that the green cable has two different ends. The “Smartphone” end goes to the iPad and the “SpikerBox” end goes to the SpikerBox.** You will no longer **hear** the spikes. Then turn on the Spike Recorder App on the iPad. Just as you did in LAB 9, use your fingertips to zoom in along the x-axis. Do you see isolated spikes (arrows in Fig.8)?

3. Measuring the frequency of spikes with the bending of the femur-tibia joint

[Background] We will next test how two types of stimuli to the leg evokes enhanced spiking activity in the nerve. We will compare the spike frequency between the resting (with no stimulus) and activated (with stimulus) states. We will first test bending the femur-tibia joint.

[Procedure]

Setup

For this experiment you will need:
1. a SpikerBox with cockroach leg prep (three electrodes penetrating the leg as shown in Fig.6)
2. an iPad that is attached to the SpikerBox via a smartphone cable
3. forceps or toothpics for bending the joint
4. a counter

Steps

1. Turn on both the SpikerBox and Spike Recorder App and observe the spontaneous activity (Fig.8).

2. Practice with the stimulation: **Using a pair of small forceps, bend the joint between the femur and the tibia** and record the activity on iPad. Do you see an increased firing rate? Find the direction of bending that elicits the most activity.

3. Once you are comfortable with the stimulation, proceed to recording.

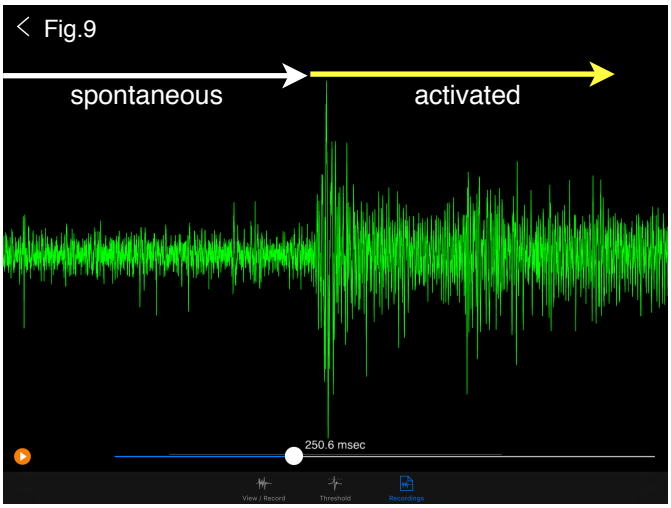
4. First, record the spike activity without any stimulus for at least 5 seconds.

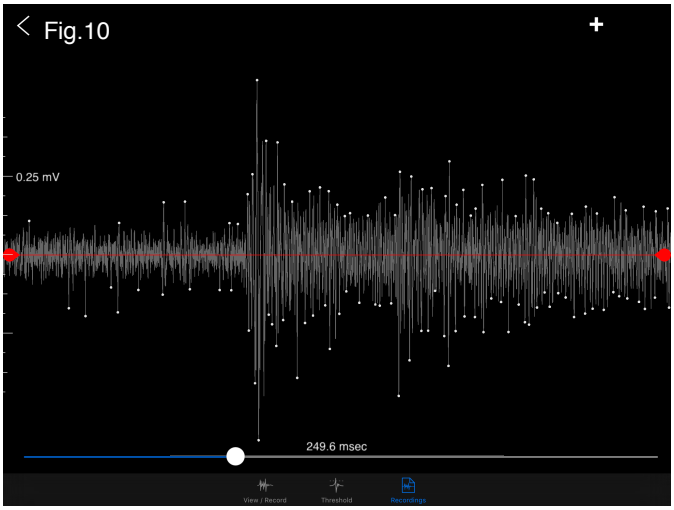
5. Bend the joint. Keep bending it for at least 3 seconds. Make sure you see robust spiking activity. Since you will not hear the sound when the SpikerBox is connected to the iPad, have the lab partner(s) watch the iPad screen as you stimulate.

6. Stop recording and replay the activity by touching the “PLAY” bouton (Fig.9).

7. Touch the “Find Spikes” bouton on the App (Fig.10). You will see white dots on peaks. Those are tentative spikes.

8. To discount noise, set a threshold by moving down the red line with your finger (Fig.11). You can now count the spikes by counting the white dots on down-going waves (see white arrows in Fig.11; red dots are discounted as a noise). Use a counter when necessary. In Fig.11, there are 4 spontaneous spikes without stimulus and 35 spikes with stimulus within the 250ms of time window. By using the scale on the left end of the graph, you can also find that the threshold amplitude is about 0.15mV. This threshold value is somewhat arbitrary, but you can be consistent by setting the same threshold in different recordings.





9. Repeat this experiments (stimulus and recording) 10 times. Fill Table 1 (next page). All members of the a group will have the same numbers in the table. Take turns in doing the stimulus and recording.

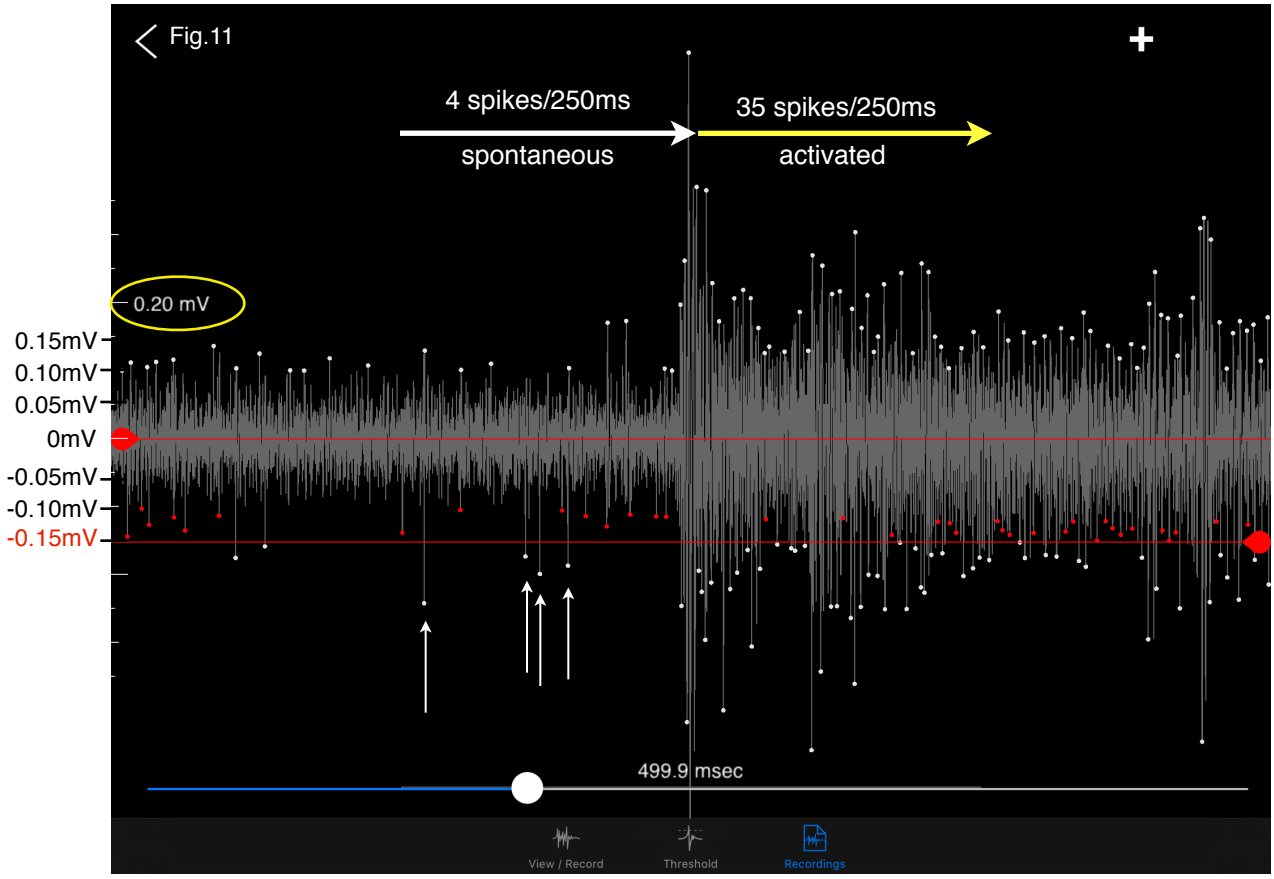


Table 1: bending	Threshold (mV)	Spike #: resting	Spike #: activated
recording #1	0.15	4	35
#2			
#3			
#4			
#5			
#6			
#7			
#8			
#9			
#10			
mean (#1-10)			

5. Measuring the frequency of spikes with the touching of a spine on the tibia

[Background] We will next test how touching a spine on the tibia evokes enhanced spiking activity in the nerve. We will compare the spike frequency between the resting (with no stimulus) and activated (with stimulus) states.

[Procedure]

Setup

For this experiment you will need:

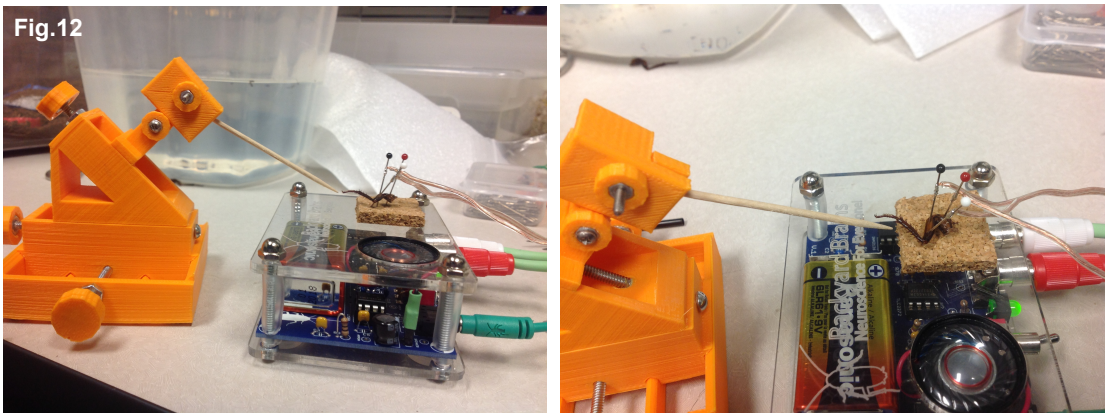
1. a SpikerBox with cockroach leg prep (three electrodes penetrating the leg as shown in Fig.6)
2. an iPad that is attached to the SpikerBox via a smartphone cable
3. Tools for stimulation:
   -toothpicks
   -a manipulator and a magnifier

Steps

1. Turn on both the SpikerBox and Spike Recorder App and observe the spontaneous activity (Fig.8).
2. Practice with the stimulation:

-Place a toothpick on the manipulator.
-Moving the knobs of the manipulator, place the end of the toothpick very close to one of the spines on the tibia of the leg (Fig.12).

Fig.12



-Using a magnifier, let the toothpick touch and move one of the spines. Make sure that you do not bend the femur-tibia joint, which will also elicit a response that you analyzed in the previous experiment. Test different directions for the displacement of the spine (see the arrows in Fig. 2 for the possible directions you want to test. You can also try directions that are shown by an arrow in Fig.2). Observe the spike activity on iPad. Do you see an increased firing rate with the touch?

-Stimulate different spines and in various directions and find which condition causes the most robust response in spike activity. You can also touch the most distal part of the leg, the tarsus.

3. Once you are comfortable with the stimulation, proceed to recording.

4. First, record the spike activity without any stimulus for at least 5 seconds.

5. Touch a spine. Keep touching it for at least 3 seconds. Make sure you see robust spiking activity right after you touch the spine. You will also notice that the roust activity does not last throughout the stimulation. This phenomenon will be further analyzed in Experiment 6: Adaptation.

6. Stop recording and replay the activity by touching the “PLAY” bouton (Fig.9).

7. Touch the “Find Spikes” bouton on the App (Fig.10). You will see white dots on peaks. Those are tentative spikes.

8. Count the spikes as you did in the previous experiments. Repeat the recording 10 times and fill in a similar table (**this will be Table 2 in the lab report**)

Table 2: touching	**Threshold (mV)**	**Spike #: resting**	**Spike #: activated**
recording #1			
#2			
#3			
#4			
#5			
#6			
#7			
#8			
#9			
#10			
mean (#1-10)			

6. Sensory adaptation

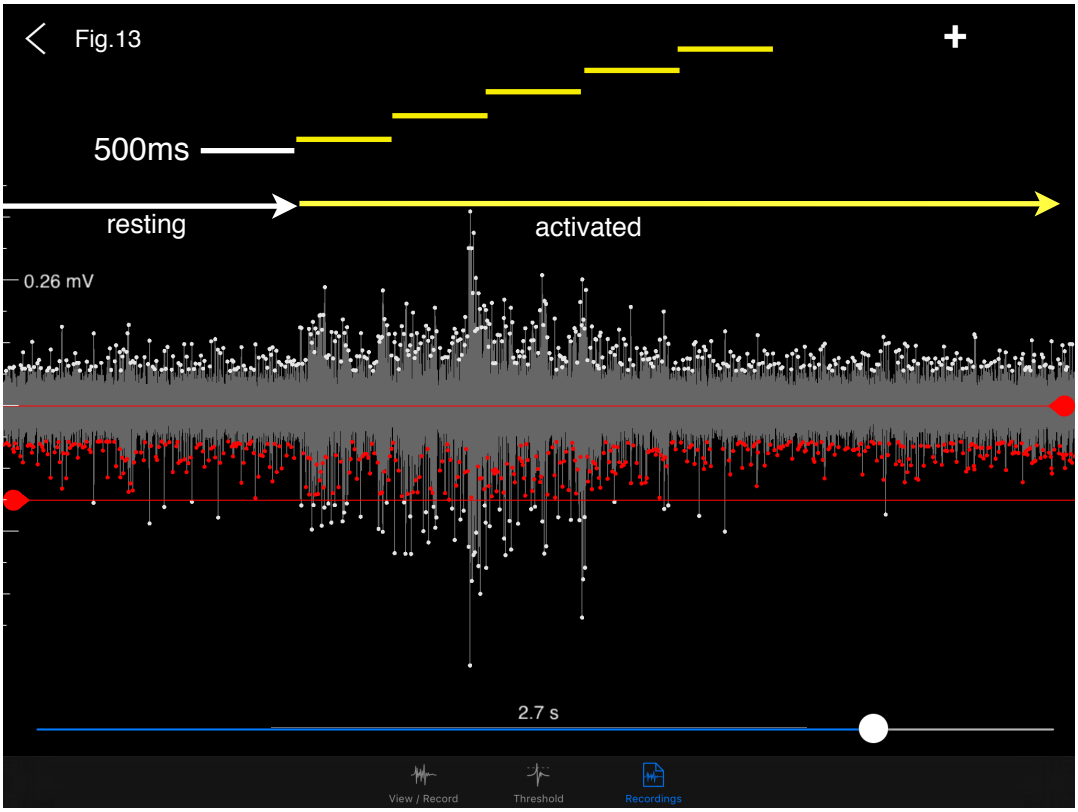
See the following website: https://www.backyardbrains.com/experiments/somatotopy

[Background] In this experiment, we will test how the response to sensory stimulus attenuates as the stimulus is continuously given. Similar to the previous experiment, we will stimulate by touching one of the spines. This time, we will keep the toothpick on the spine and record the spiking activity to determine how the response changes over time.

[Procedure]

This is similar to the previous experiment -Touch a spine on the tibia or the femur-. **This time, pay a special attention so that the end of the toothpick stays at the same position and keeps touching the spine that initially elicits a strong response in nerve activity.** Observe what happens to the enhanced spiking. Does it last infinitely or does it attenuate over time?

Once you make the initial observation, record the activity and count the spines in in 500ms intervals (Fig. 13).



500ms right before the onset of stimulus (spontaneous spikes)

500ms right after the onset of stimulus

next 500ms

next 500ms

.....

Fill in the table 3 (numbers were taken from the graph above).

Repeat this three times using the same spine and find out how many seconds it take for the spike frequency to go back to the level before the stimulus. Do not stimulate the same spine without any resting period. Wait at least one minute before you apply the toothpick again.

Table 3: adaptation	record 1	record 2	record 3
Spike # before stim	2		
Spike #: first 250ms	14		
Spike #: second 250ms	23		
Spike #: third 250ms	17		
Spike #: fourth 250ms	5		
Spike #: fifth 250ms	2		

[in-lab discussion points]

1. What is a voltage? When a voltage is reported, is it an absolute number or is it a differential value?
2. What do you think would happen if you simply reversed your white and black electrodes? Will it change the wave form?
3. Why do you think the adaptation occurs? What mechanisms can adapt to continued stimuli?

[Lab report]

Fill in Table 1 (1.4 points), Table 2 (1.4 points) and Table 3 (1.2 points)

LAB 11: Human cadaver lab

Attendance at this lab is optional and submitting a lab report is not required.

Make sure the instructors knows your intention to attend the lab.

Before coming to the cadaver lab, you need to do two things listed below. Otherwise, you will NOT be admitted to the lab.

1) Watch the video online (http://mediamill.cla.umn.edu/mediamill/embed/18412) that explains what you should know to participate. You will find more general information about the Anatomy Bequest Program on their web site (Go to the website of Anatomy Bequest Program (http://www.bequest.umn.edu). We may ask you about the content of the video to verify that you actually watched it.

2) Complete the form attached at the end of this manual, and turn it in when you come to LAB 11. If you do not turn in this form, you will not be allowed to participate.

Objectives:
1. Understand the anatomy of the meninges
2. Understand the anatomical relationship between the brain and the rest of the body, particularly:
   a. major arteries and veins
   b. cranial nerves
   c. major parts of the brain relative to the parts of head

Location:
Human anatomy lab (5-167 Jackson Hall)

Date and time: Same as your regular lab, but we will split each group into two smaller groups. Each demonstration will take 30-45 min.

Materials:
human cadavers (prepared by Human Anatomy teaching staff)

Note:
For most of you, it will be your first experience seeing a human cadaver. If you start to feel uncomfortable after entering the lab, which is a normal reaction, take a rest on a chair in the room. If this does not help, feel free to leave the room. Many of our medical students have this reaction on the first day of anatomy class.

Procedure:

1. Each group of students (8-10) will be invited in the lab. Put your backpack on the cart provided.
2. An Instructor will introduce you to the cadaver, and discuss the medical history of the person.
3. The instructor will remove the skin and skull to expose the brain.
4. The brain will be lifted out of the skull and the instructor will show you the base of the skull. The skull base has three parts: anterior, middle and posterior cranial fossa (Fig.2). The instructor will also show you the pituitary.
   http://www.youtube.com/watch?v=y1ExSUGUsU0
5. The instructor will show major foramena (holes) of the cranium through which the medulla, major arteries and cranial nerves are connected to the rest of the body.
   http://www.youtube.com/watch?v=9vJ-tEyD68k
   http://www.youtube.com/watch?v=T1cebHMv8oI
6. The instructor will show the dura, superior sagittal sinus and cavernous sinus.

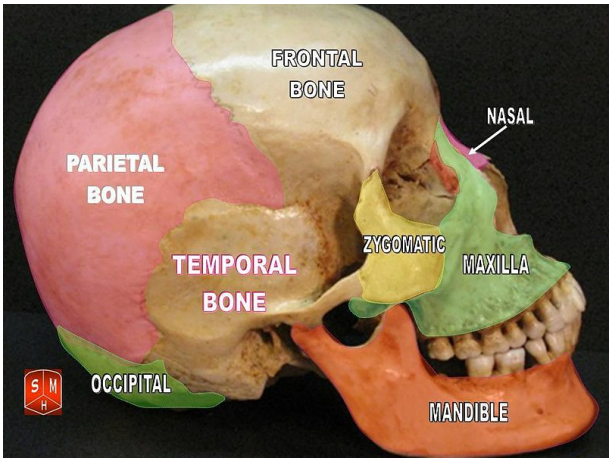
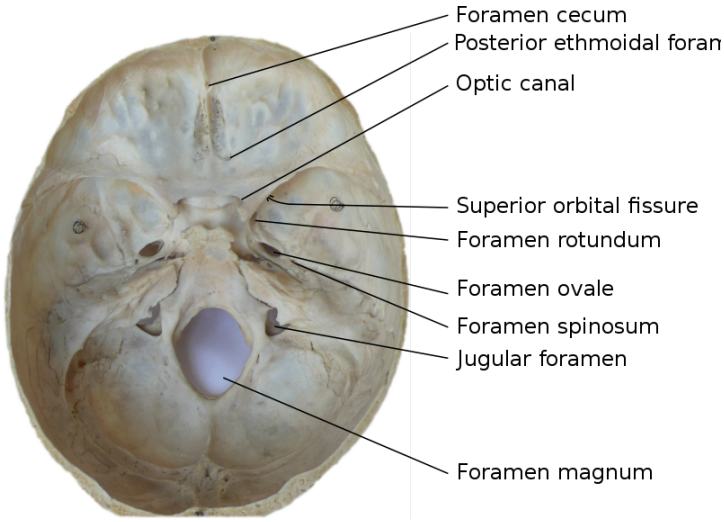
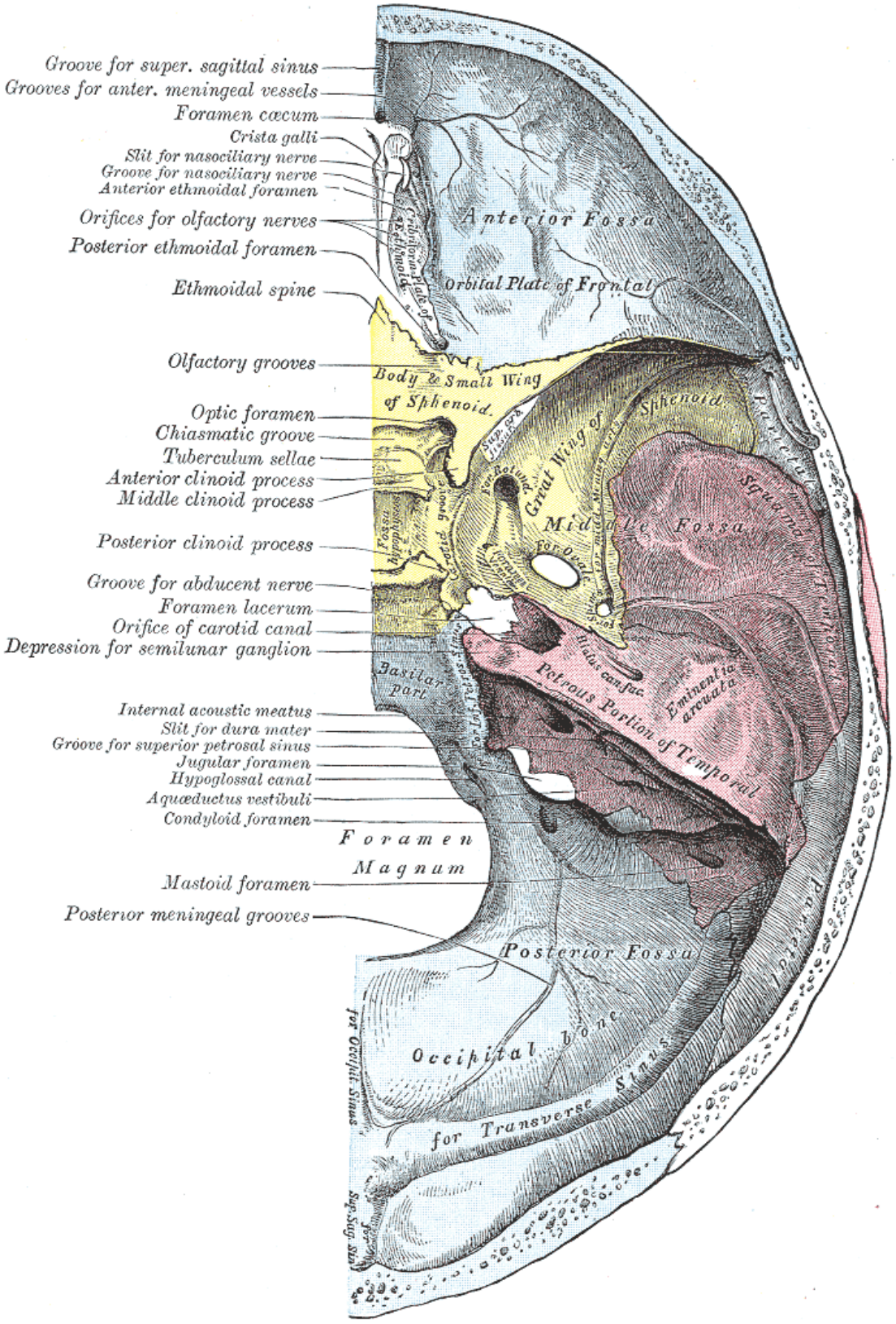
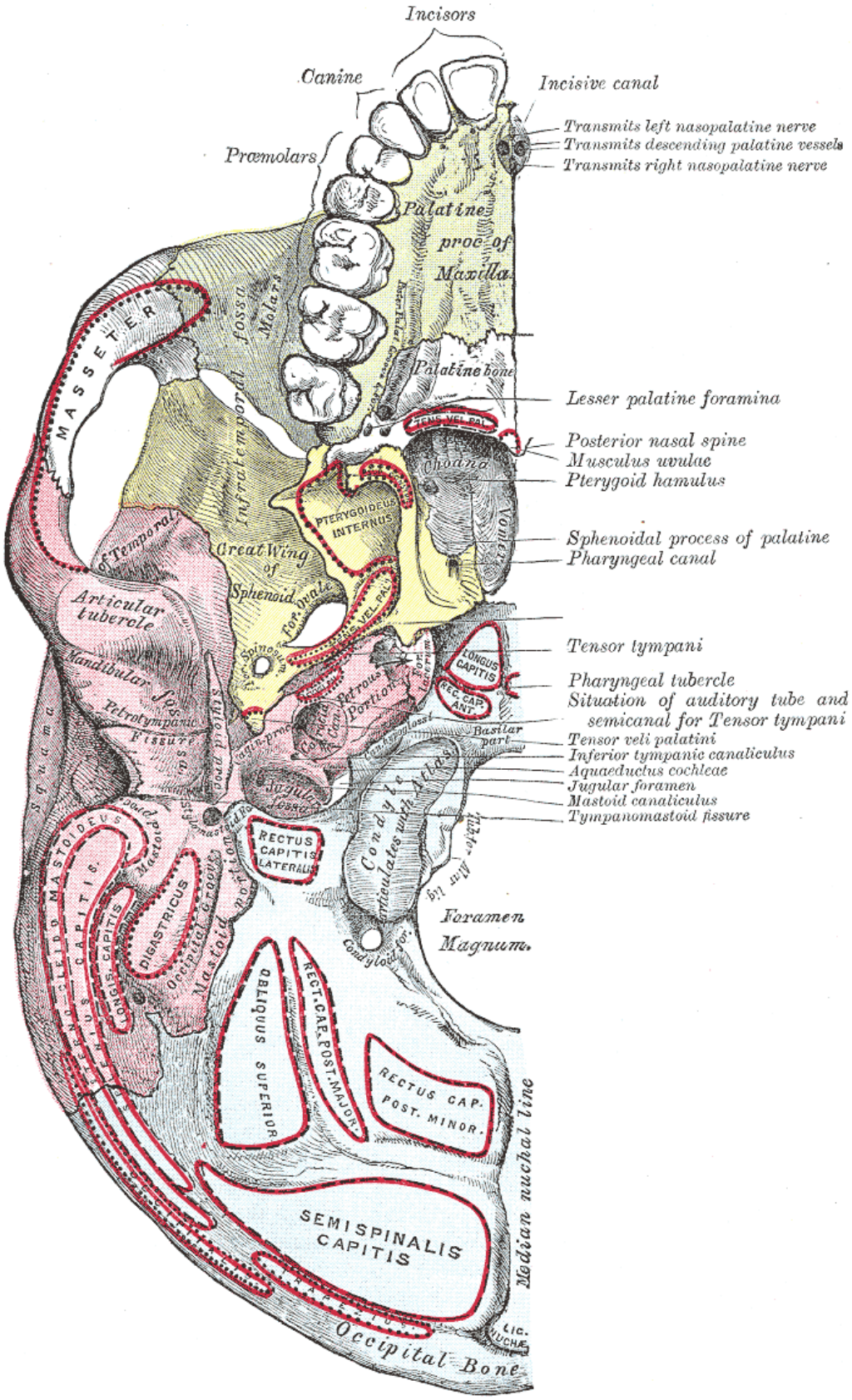


Fig.1

Fig.2

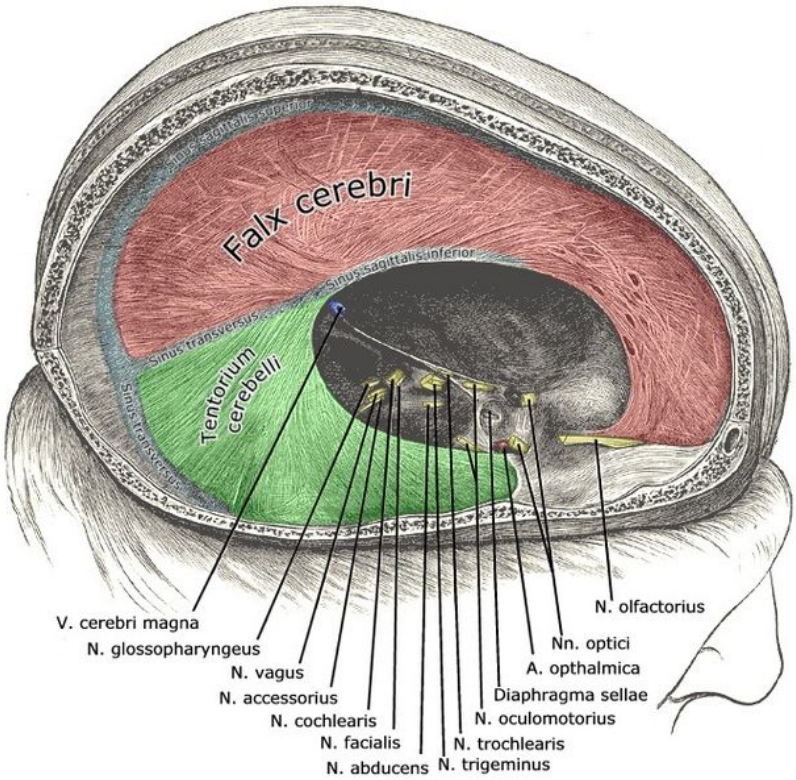


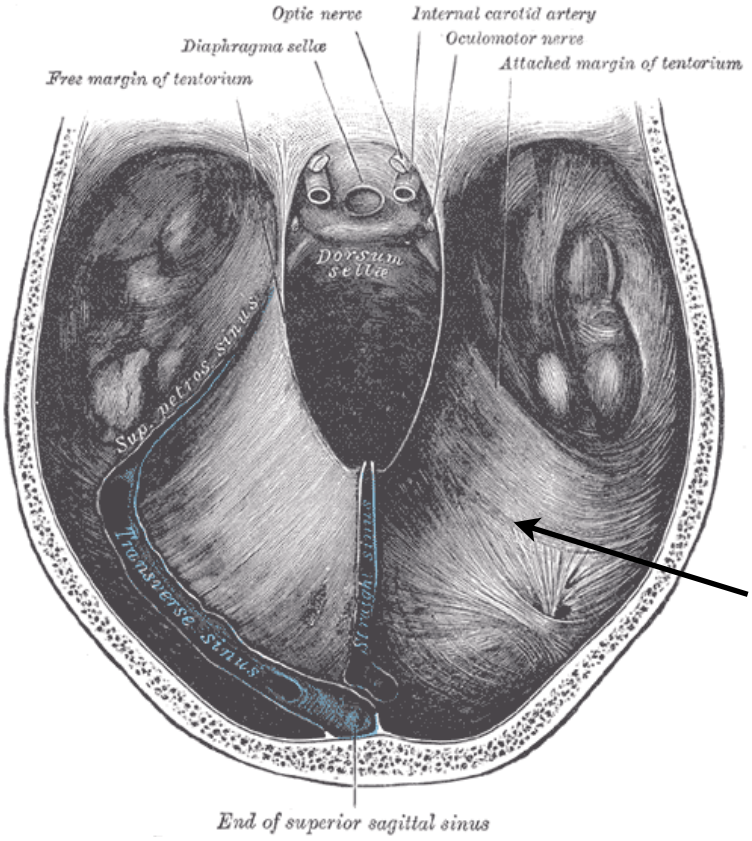

Groove for super. sagittal sinus
Grooves for anter. meningeal vessels
Foramen cæcum
Crista galli
Slit for nasociliary nerve
Groove for nasociliary nerve
Anterior ethmoidal foramen
Orifices for olfactory nerves
Posterior ethmoidal foramen
Ethmoidal spine
Olfactory grooves
Optic foramen
Chiasmatic groove
Tuberculum sellae
Anterior clinoid process
Middle clinoid process
Posterior clinoid process
Groove for abducent nerve
Foramen lacerum
Orifice of carotid canal
Depression for semilunar ganglion
Internal acoustic meatus
Slit for dura mater
Groove for superior petrosal sinus
Jugular foramen
Hypoglossal canal
Aquæductus vestibuli
Condyloid foramen
Foramen Magnum
Mastoid foramen
Posterior meningeal grooves
Anterior Fossa
Orbital Plate of Frontal
Body & Small Wing of Sphenoid.
Great Wing of Sphenoid.
Middle Fossa
Squama of Temporal
Petrous Portion of Temporal
Basilar part
Posterior Fossa
Occipital bone
for Transverse Sinus


Incisors
Canine
Incisive canal
Præmolars
Transmits left nasopalatine nerve
Transmits descending palatine vessels
Transmits right nasopalatine nerve
Palatine proc. of Maxilla
Palatine bone
Lesser palatine foramina
Posterior nasal spine
Musculus uvulae
Pterygoid hamulus
Sphenoidal process of palatine
Pharyngeal canal
Tensor tympani
Pharyngeal tubercle
Situation of auditory tube and semicanal for Tensor tympani
Tensor veli palatini
Inferior tympanic canaliculus
Aquaeductus cochleae
Jugular foramen
Mastoid canaliculus
Tympanomastoid fissure
Foramen Magnum.
Median nuchal line
Occipital Bone
MASSETER
Temporal
Articular tubercle
Mandibular fossa
Petrotympanic Fissure
Great Wing of Sphenoid
Infratemporal fossa
Molars
PTERYGOIDEUS INTERNUS
TENS. VEL. PAL.
LONGUS CAPITIS
REC. CAP. ANT.
RECTUS CAPITIS LATERALIS
OBLIQUUS SUPERIOR
RECT. CAP. POST. MAJOR
RECTUS CAP. POST. MINOR
SEMISPINALIS CAPITIS
DIGASTRICUS
STERNO-CLEIDO-MASTOIDEUS
SPLENIUS CAPITIS
LONGUS CAPITIS
LIG. NUCHÆ

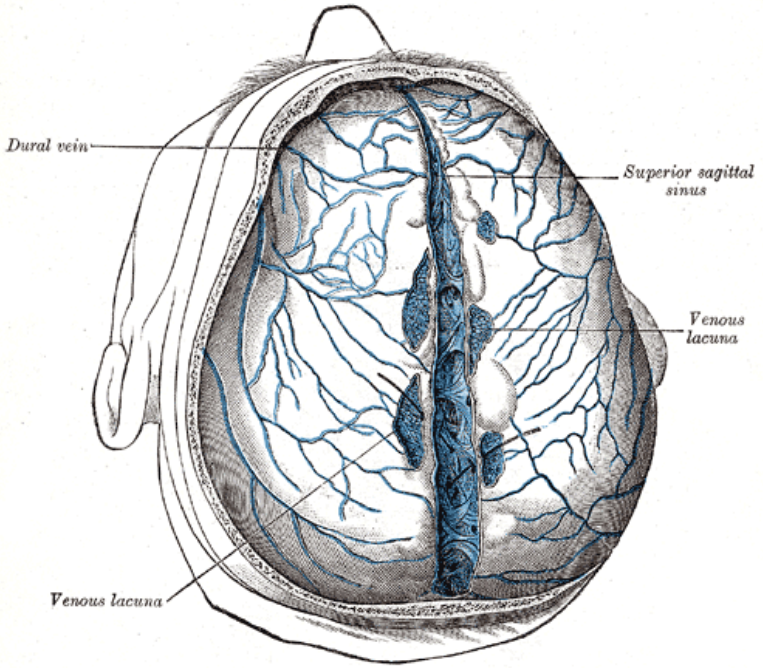
The **falx cerebri**, so named from its sickle-like form, is a strong, arched fold of the dura mater that descends vertically in the longitudinal fissure between the cerebral hemispheres.

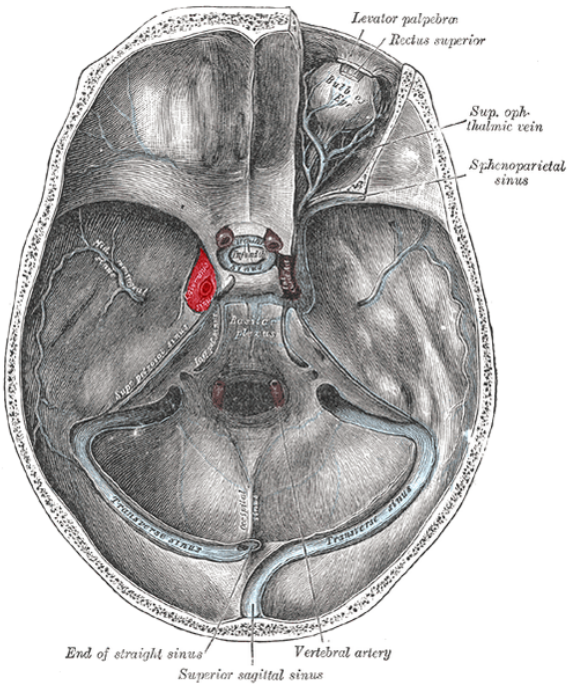
The **tentorium cerebelli** covers the superior surface of the cerebellum, and supports the occipital lobes of the brain.





tentorium cerebelli





Superior sagittal sinus is at the top of the falx cerebri (part of the dura mater; see next page). It allows blood to drain from the cerebral hemispheres. Cerebrospinal fluid drains through arachnoid granulations into the superior sagittal sinus and is returned to venous circulation.

Cavernous sinus (red on left) has bone on its medial side. It is filled with venous blood that returns to the body (via internal jugular vein), but also contains the following structures:

oculomotor nerve
trochlear nerve
trigeminal ganglion
internal carotid artery