Brain Histology and mouse models

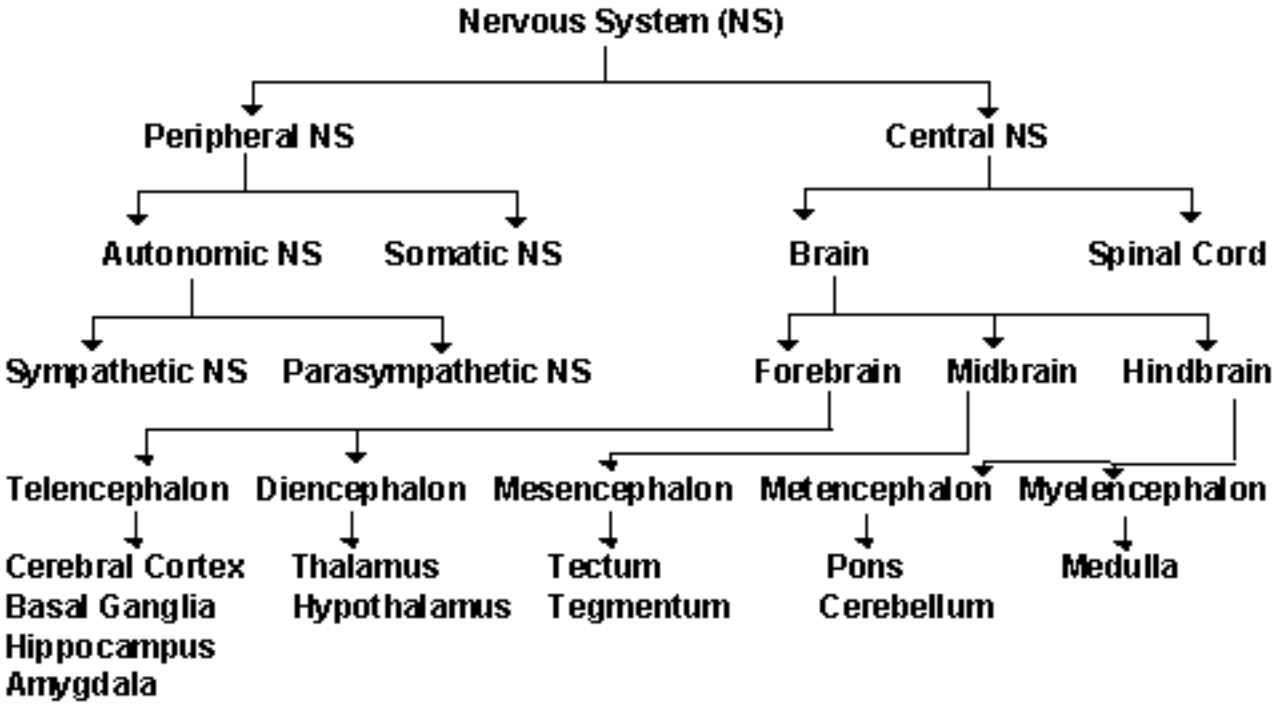
2018 Spring

Brain and Spinal Cord

Central Nervous System

Peripheral Nervous System

Human

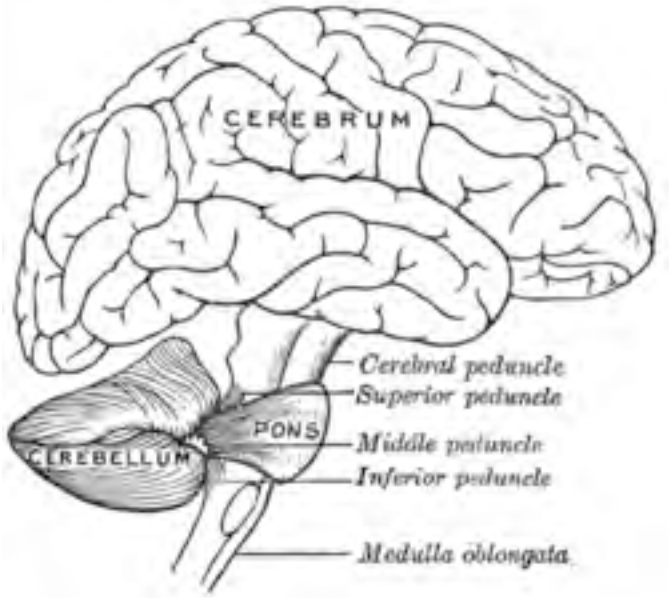


Brain:

Cerebrum

Cerebellum

Brain Stem



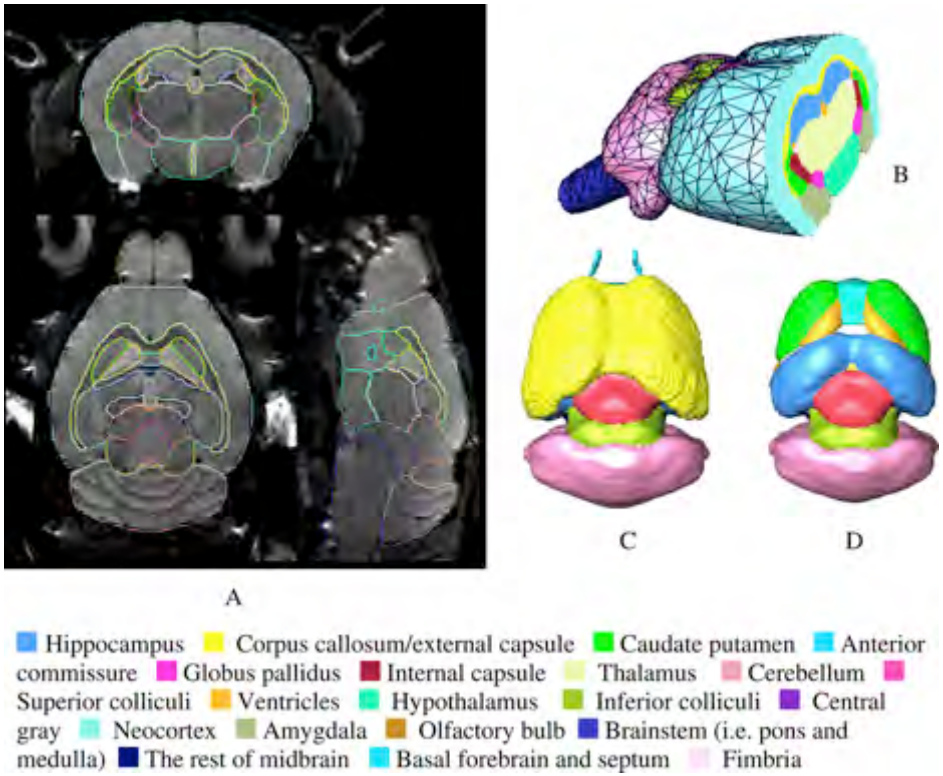
In mice:

Olfactory lobes

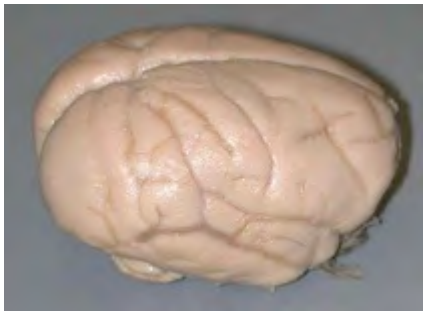
Cerebrum

Cerebellum

Brain Stem



Human



Monkey

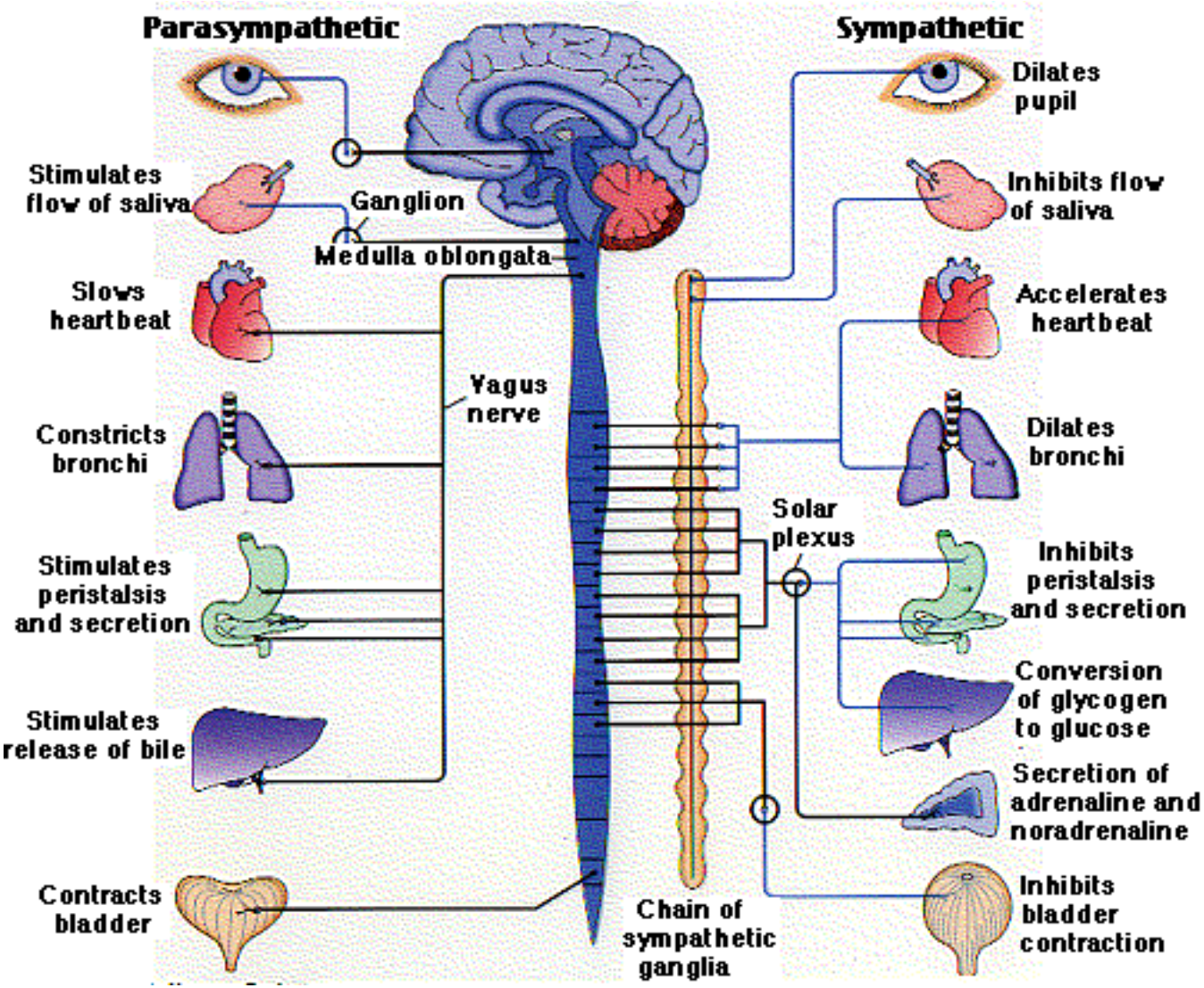
Cat

Rat

Frog

What do each of these lobes do?

* **Frontal Lobe- associated with reasoning, planning, parts of speech, movement, emotions, and problem solving**
* **Parietal Lobe- associated with movement, orientation, recognition, perception of stimuli**
* **Occipital Lobe- associated with visual processing**
* **Temporal Lobe- associated with perception and recognition of auditory stimuli, memory, and speech**


Parasympathetic
Sympathetic
Dilates pupil
Stimulates flow of saliva
Ganglion
Medulla oblongata
Inhibits flow of saliva
Slows heartbeat
Accelerates heartbeat
Vagus nerve
Constricts bronchi
Dilates bronchi
Solar plexus
Stimulates peristalsis and secretion
Inhibits peristalsis and secretion
Conversion of glycogen to glucose
Stimulates release of bile
Secretion of adrenaline and noradrenaline
Contracts bladder
Chain of sympathetic ganglia
Inhibits bladder contraction

The organs (the "viscera") of our body, such as the heart, stomach and intestines, are regulated by a part of the nervous system called the autonomic nervous system (ANS). The ANS is part of the peripheral nervous system and it controls many organs and muscles within the body. In most situations, we are unaware of the workings of the ANS because it functions in an involuntary, reflexive manner. For example, we do not notice when blood vessels change size or when our heart beats faster.

The ANS regulates:

•Muscles in the skin (around hair follicles; smooth muscle)
around blood vessels (smooth muscle), in the eye (the iris; smooth muscle) in the stomach, intestines and bladder (smooth muscle)
of the heart (cardiac)
***Glands**

The ANS is divided into three parts:

* **The sympathetic nervous system**
* **The parasympathetic nervous system**
* **The enteric nervous system.**

The Mouse Brain Library

http://www.mbl.org/mbl_main/mbl_procedure.html

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The Mouse Brain Library

Home | Library | iScope | Brain Atlas | Micrographs | Procedures | Movies | Databases

Procedures

MBL Training Manual
Mouse Brain Library Training Manual - Detailed Protocol

Mouse Brain Atlas Tutorial
Information, images, and HTML for creating an atlas of your own

Micrograph Imaging Tutorial
Procedure used to scan the micrographs and acquire the data on this site.

The Mouse Brain Library

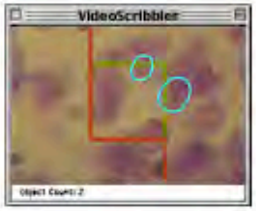
http://www.mbl.org/mbl_main/mbl_procedure.html

Most Visited Getting Started Latest Headlines

The Mouse Brain Library

MBL Slide Library Tutorial
Step-by-step instructions for creating a slide database in FileMaker.

iScope Internals Manual
A tutorial on the iScope is available here.



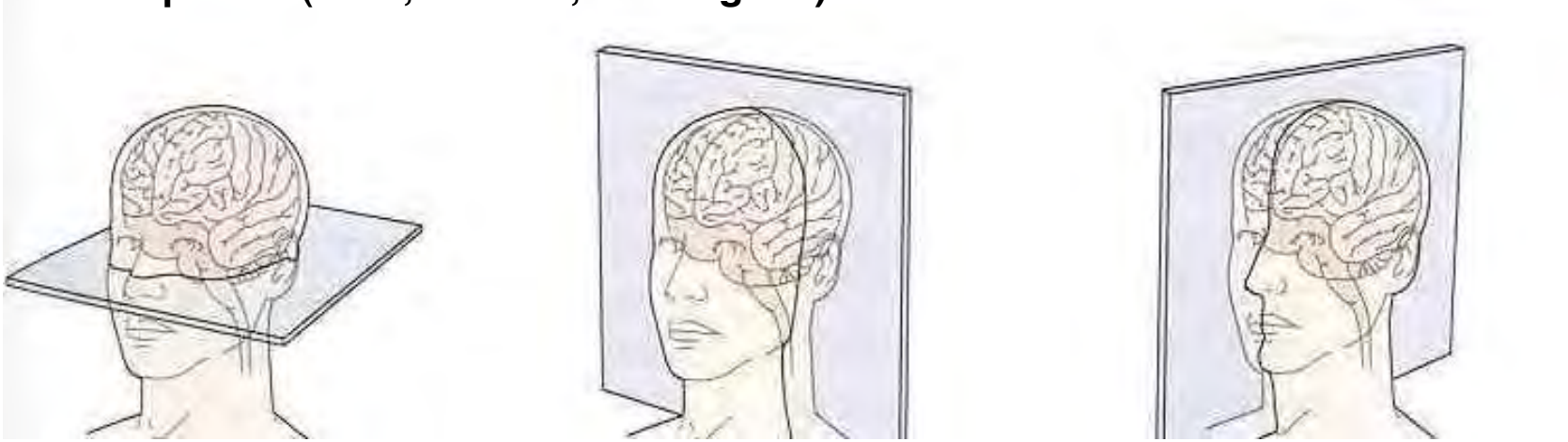
VideoScribbler for Mac
A program for the Macintosh that lets you draw on top of a live video source. VideoScribbler was developed for cell counting.

Point Counting Macros
A set of time saving point counting macros for Object Image.

Tissue Processing Procedure
Information on our tissue processing procedure.

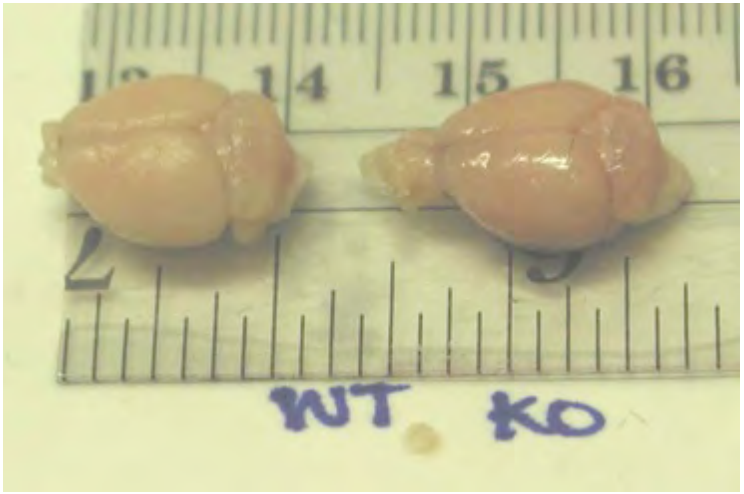
Three directional planes of the brain: *rostral/caudal*, *dorsal/ventral*, and *medial / lateral*. When sectioning (cutting) the brain, which planes are visible is determined by the type of section.

In the sagittal section (which is made parallel to the midline, dorsal to ventral) the rostral/caudal and dorsal/ventral planes can be seen. In the coronal or cross section (made perpendicular to the midline, as if you're slicing a loaf of bread) the medial/lateral and dorsal/ventral planes can be seen. The image below shows the 3 different planes (axial, coronal, and sagittal) in which a brain can be sectioned:



Axial, coronal, sagittal

www-psych.stanford.edu/~kalina/BB



We generally examine organs from a

FULLY back-crossed set of animals, minimum of:

6 males, wild type, littermate controls

6 females, wild type, littermate controls

6 males, genetically altered

6 females, genetically altered

12 initial and then 12 more follow up

Weights and measurements may become important

Examination of one set of organs is NOT sufficient

Need to analyze at least 6 sets of organs

To determine if differences observed are statistically significant

http://www.mbl.org/mbl_main/atlas.html

Google IWDC Conten...ent Portal HistoShared University o...a, San Diego UCSD ACS / ... Operations Apple

Home Library iScope Brain Atlas Micrographs Procedures Movies Databases

Mouse Brain Atlases

Coronal

C57BL/6J - DBA/2J

Horizontal

C57BL/6J - DBA/2J - A/J

News:

6/24/05 - Atlas of Developing Mouse Brain Gestational (Embryonic) Day 12: Schambra, Annotations Complete

09/17/04 - Atlas of Developing Mouse Brain Embryonic Day 12: Schambra, in progress

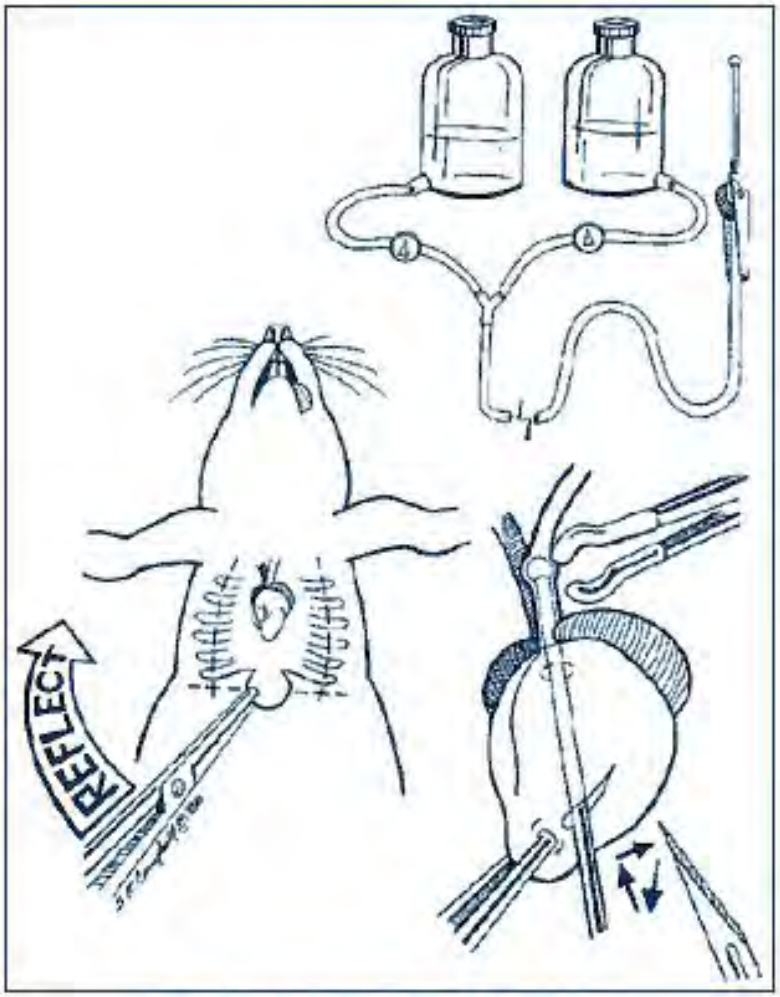
01/6/04 - How to Make your own Atlas in 10 Easy Steps

08/2/02 - Horizonal A/J Atlas. This is a high resolution (2.6µm/pixel) atlas in 8-bit color.

07/16/01 - Horizontal DBA/2J Atlas. This is a high resoution (3.5 µm/pixel) atlas in 8-bit color.

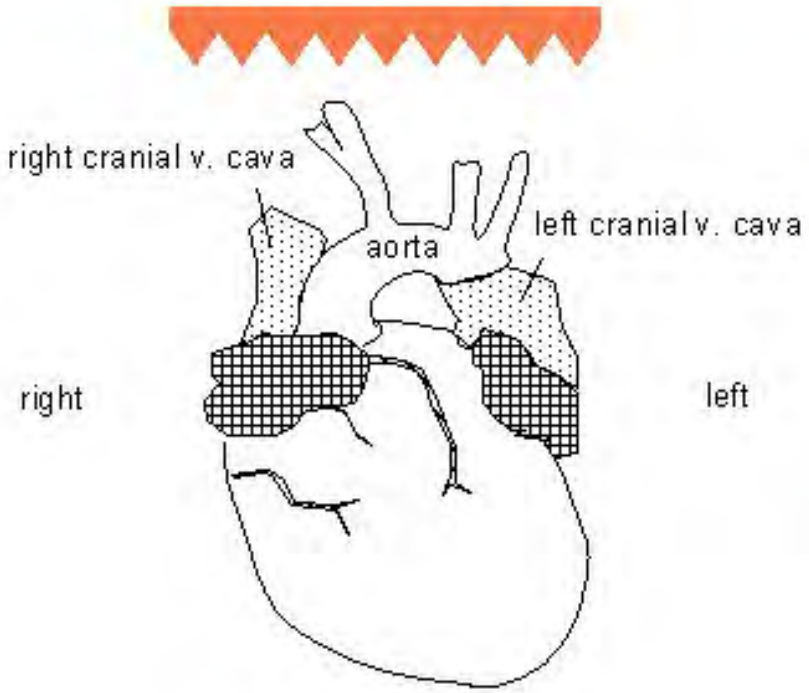
6/00 Mouse Brain Atlas Tutorial and related files added. This tutorial gives detailed instructions

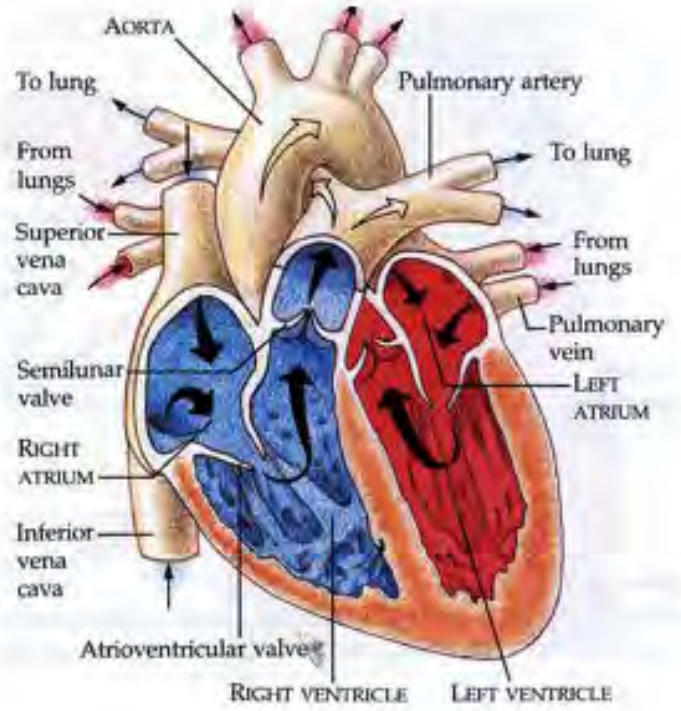
http://www.neurosciencesassociates.com/



Perfusion Setup Diagram

Perfusion setup to perfuse the whole animal





Perfusion of the whole animal

Via the left ventricle is recommended for optimal examination of the brain and in certain other situations

Important points to remember for mouse brain histology :

--Perfusion fixation is important to avoid artefacts, such as “dark neurons”

--Do not leave in 70% alcohol for longer than 24 hours, to avoid vacuole artefact

--both erythrocytes and degenerating neurons are autofluorescent.

--To avoid hypostatic congestion in multiple tissues and to improve the quality of exsanguination, animals should be anesthetized as closely to the time of euthanasia as is practical.

Preparation and analysis of the central nervous system

http://tpx.sagepub.com/content/39/1/58.full

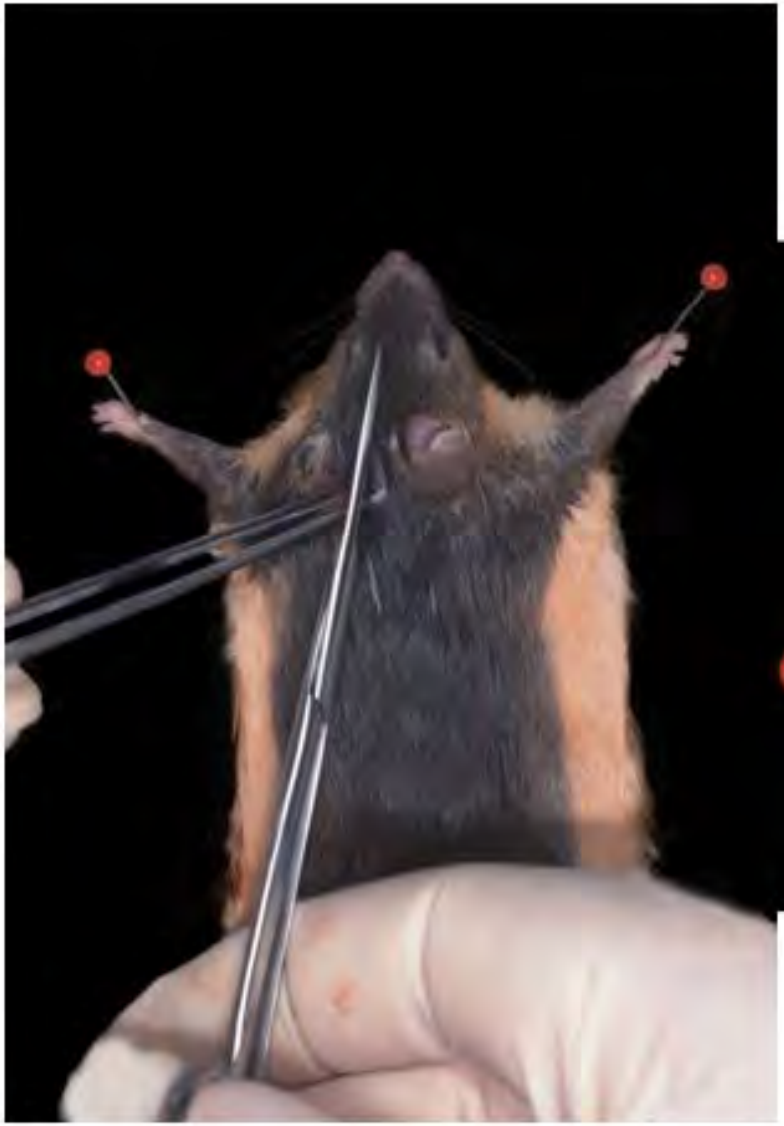
Examination of the mouse Brain

Planes of section

Cell types within the brain

Special stains

Peripheral Nervous System



www.eulep.org

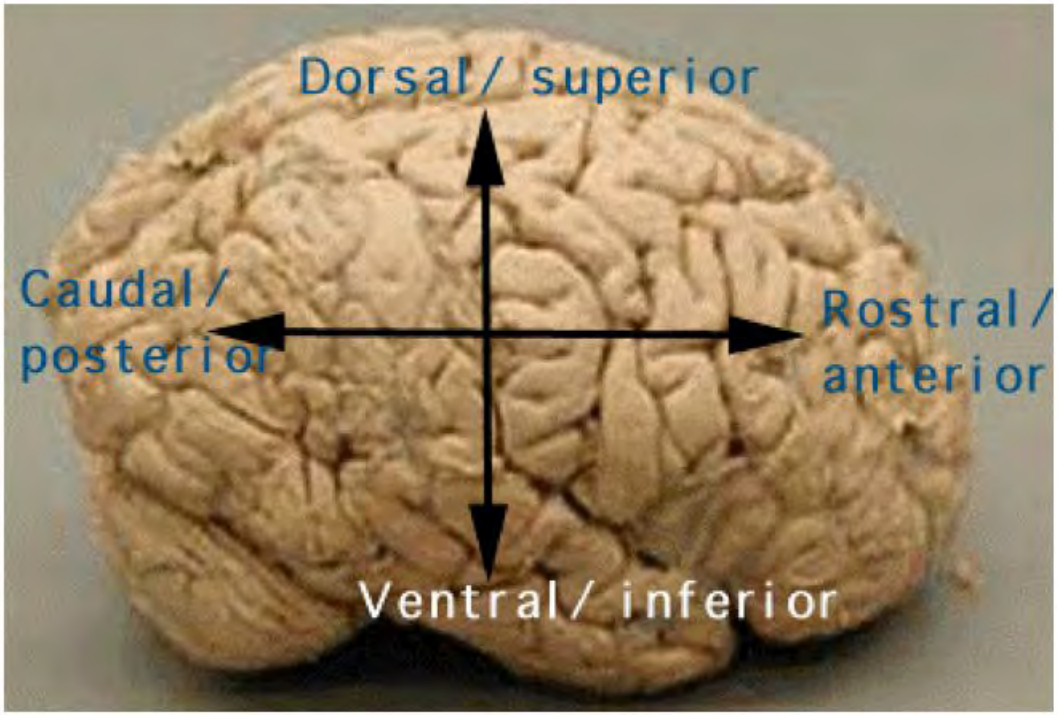
Opening the skull to remove the mouse brain

Frozen tissues:

1. must be frozen using the correct conditions
2. Must be stored at minus 80 or at liquid nitrogen temperatures
3. Morphology is not the best
4. Usually better for immuno assays

Fixed tissues:

1. Must be fixed as thin slices
2. In at least 10 volumes of fixative
3. Must be processed after 24 hours of fixation if immunoassays are to be done on the processed paraffin sections
4. If fixed and processed optimally, results in the best morphology
5. Immunoassays are a little challenging

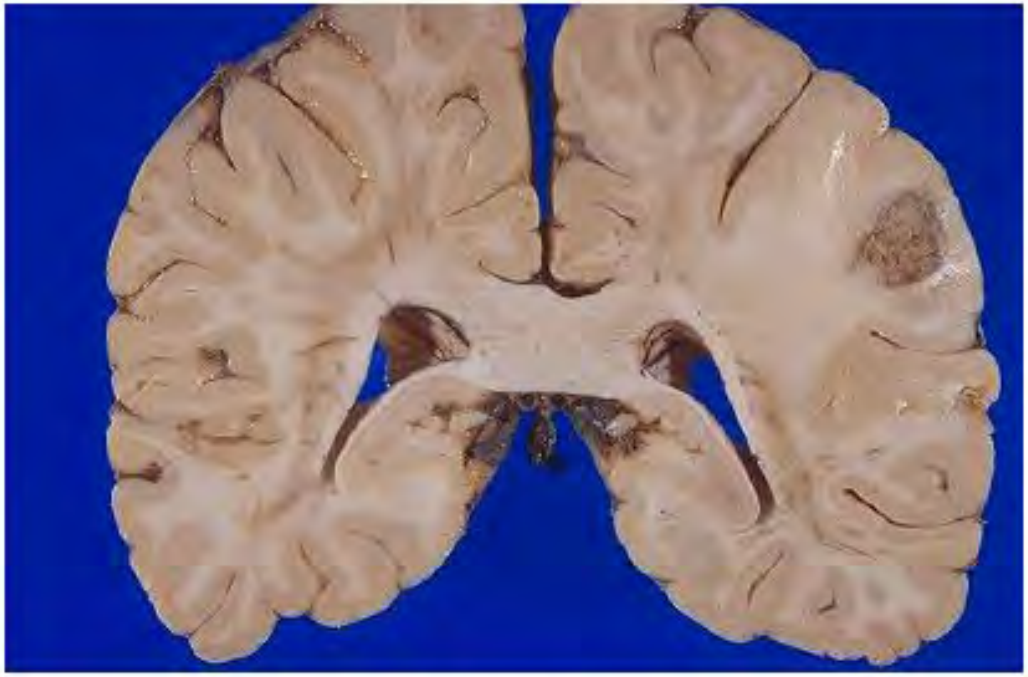


Human Brain

With gyri and sulci

Mouse Brain

lissencephalic



Coronal section Human Brain with pathology

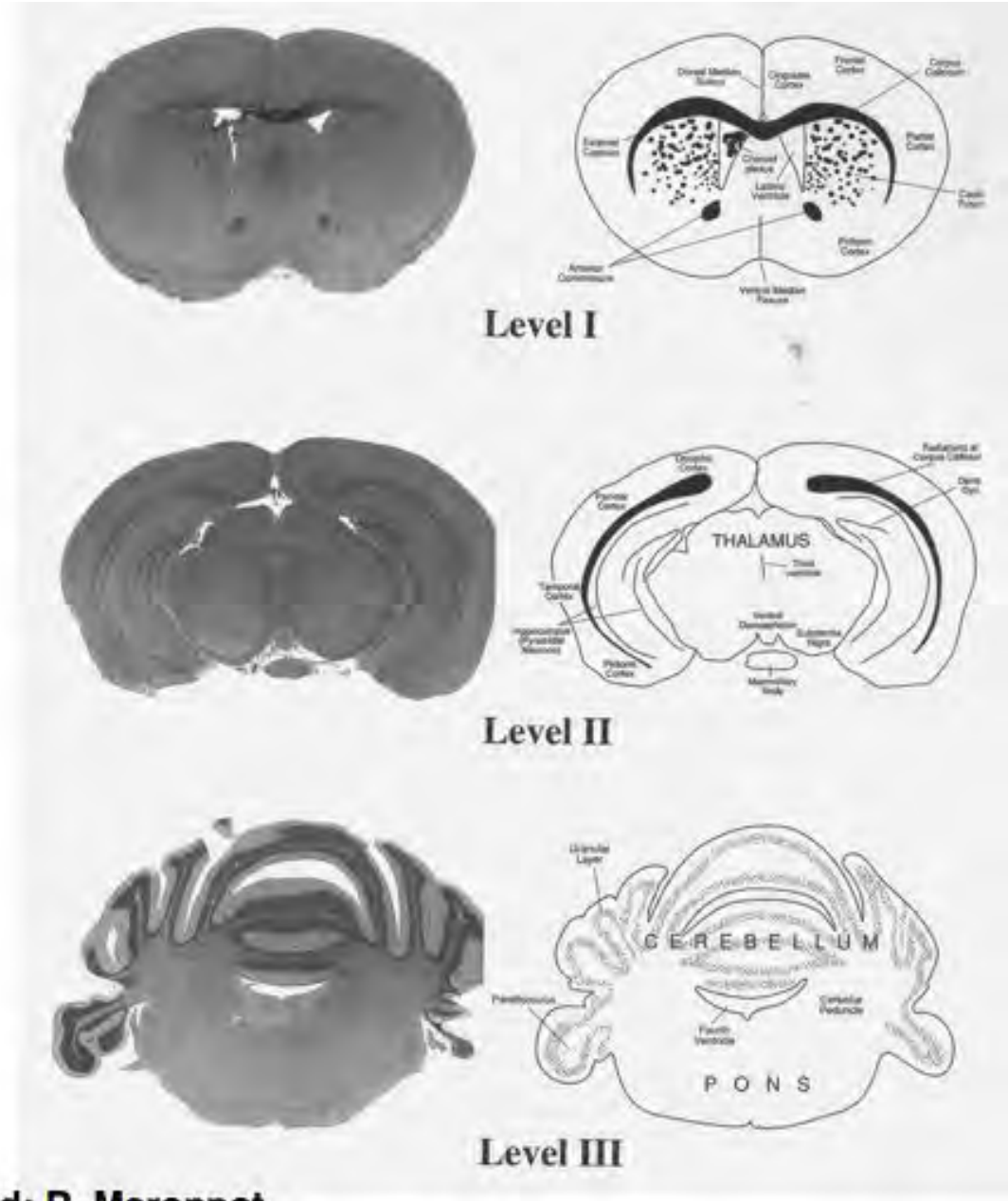
http://www-medlib.med.utah.edu/WebPath/

Coronal section Mouse Brain

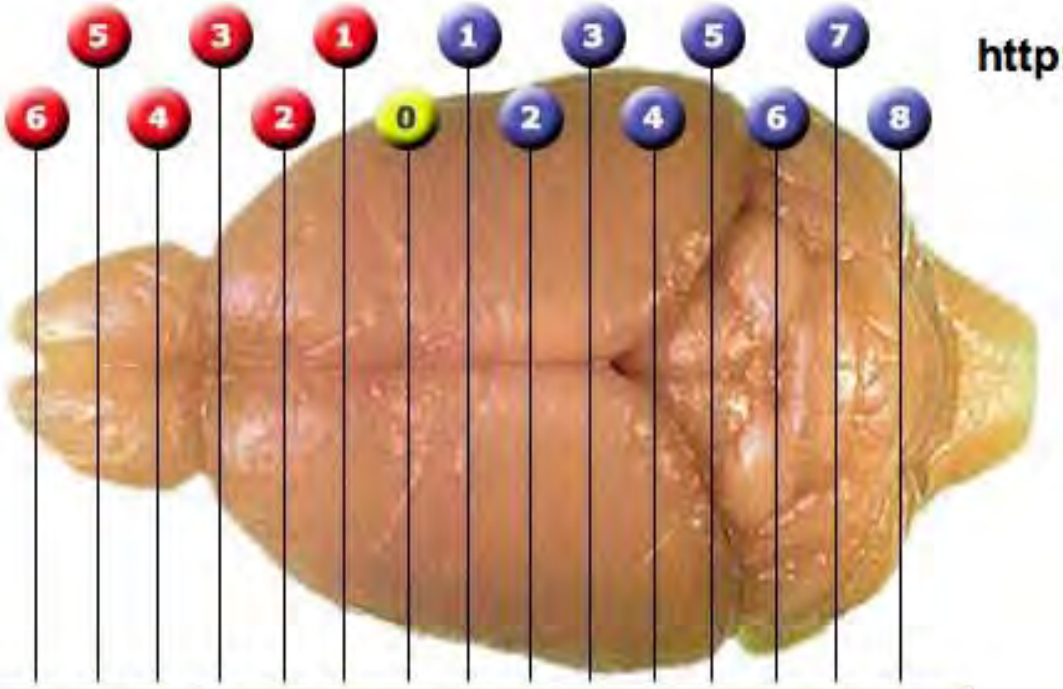
www.mbl.org

Figure 2: Ventral surface of the mouse brain. Black lines indicate the position of the sections shown in Figure 1.

gure 3: Mid-sagittal section of the mouse brain.



From: Pathology of the mouse Ed: R. Maronpot

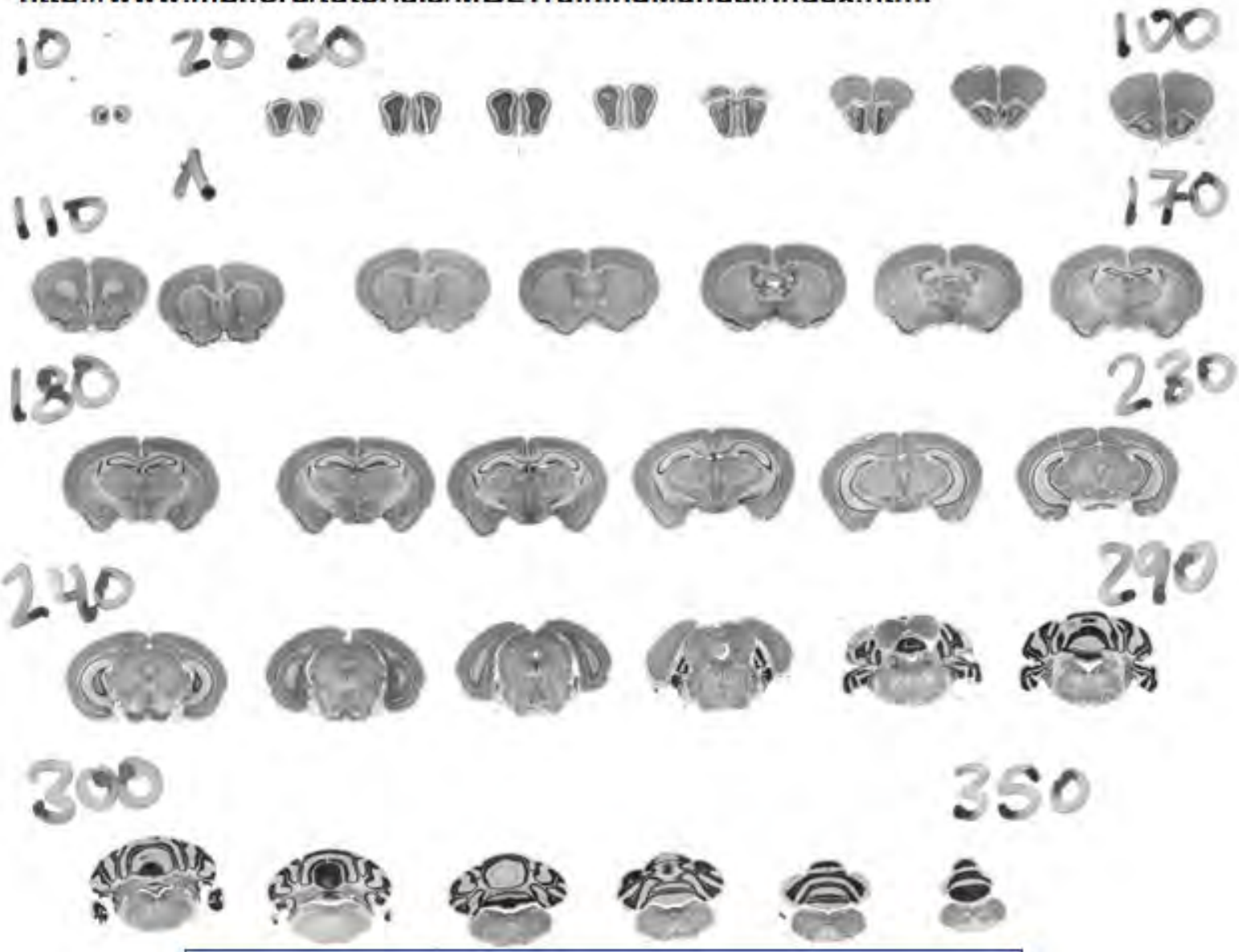


http://www.mbl.org/

Coronal sections through the mouse brain

The markers above are spaced 1 mm apart and correspond to levels of the Atlas. The brain is that of a 51-day-old C57BL/6J male with a body weight of 20.2 gm and a brain weight (fixed) of 477 mg . A brain such as this one contains approximately 75 million neurons, 23 million glial cells, 7 million endothelial cells associated with blood vessels, and 3 to 4 million miscellaneous pial, ependymal, and choroid plexus cells

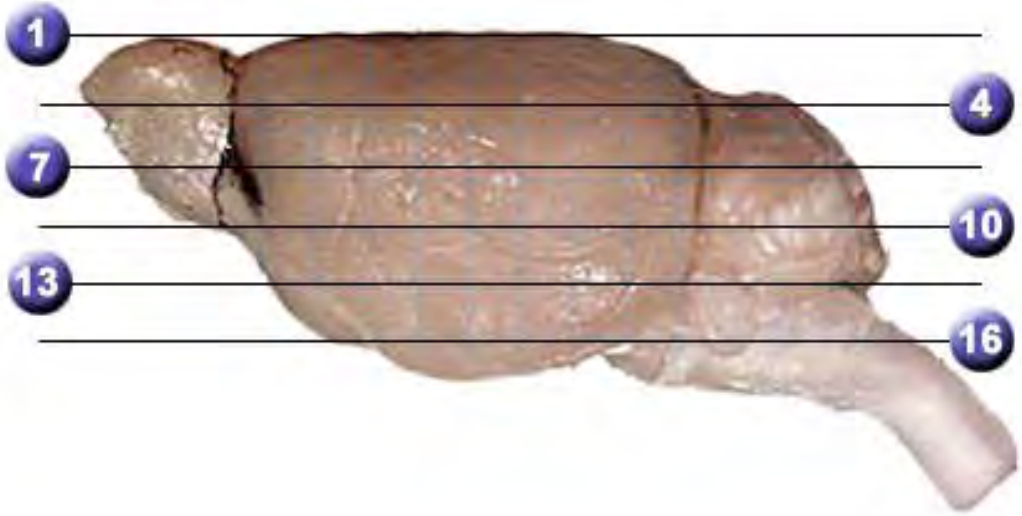
http://www.mbl.org/tutorials/MBLTrainingManual/index.html

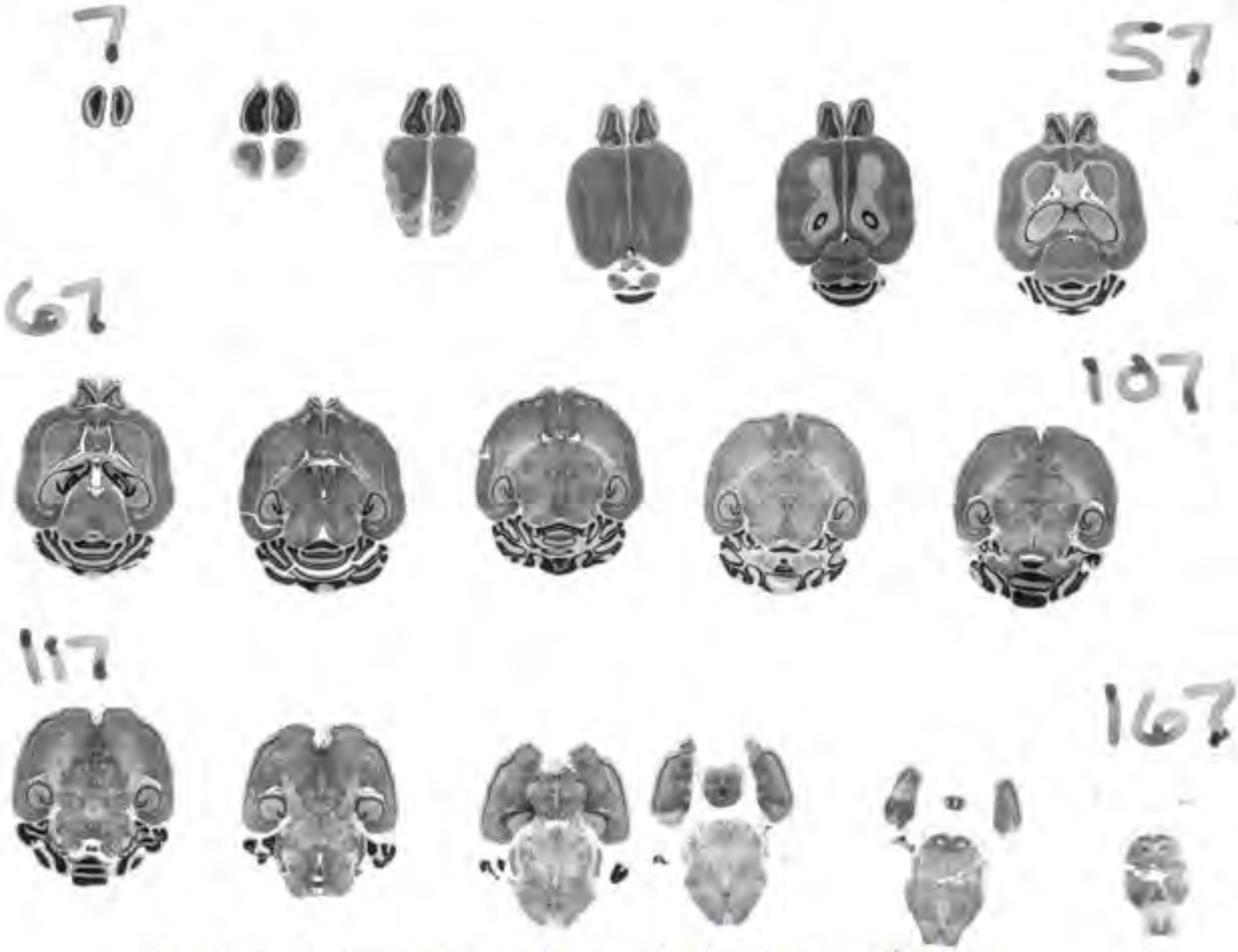


Coronal sections through the mouse brain

http://www.mbl.org/

Horizontal sections through the mouse brain

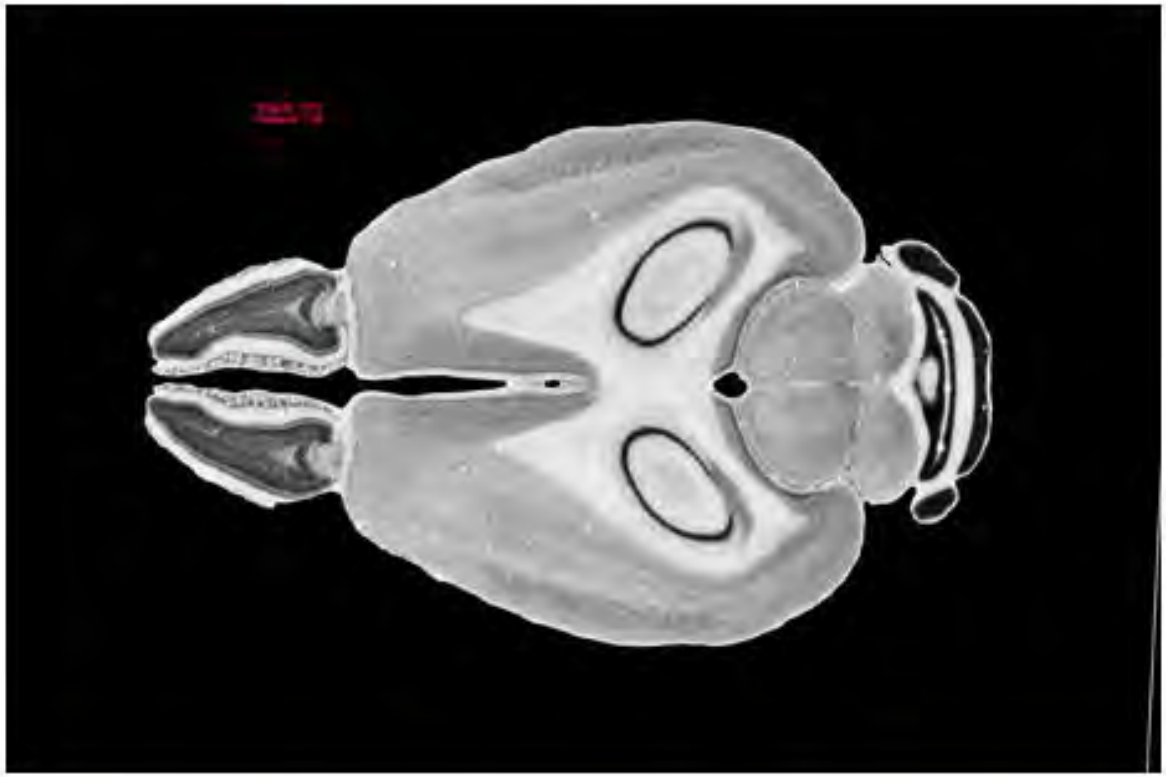




Horizontal sections through the mouse brain

Section 4 horizontal

http://www.mbl.org/

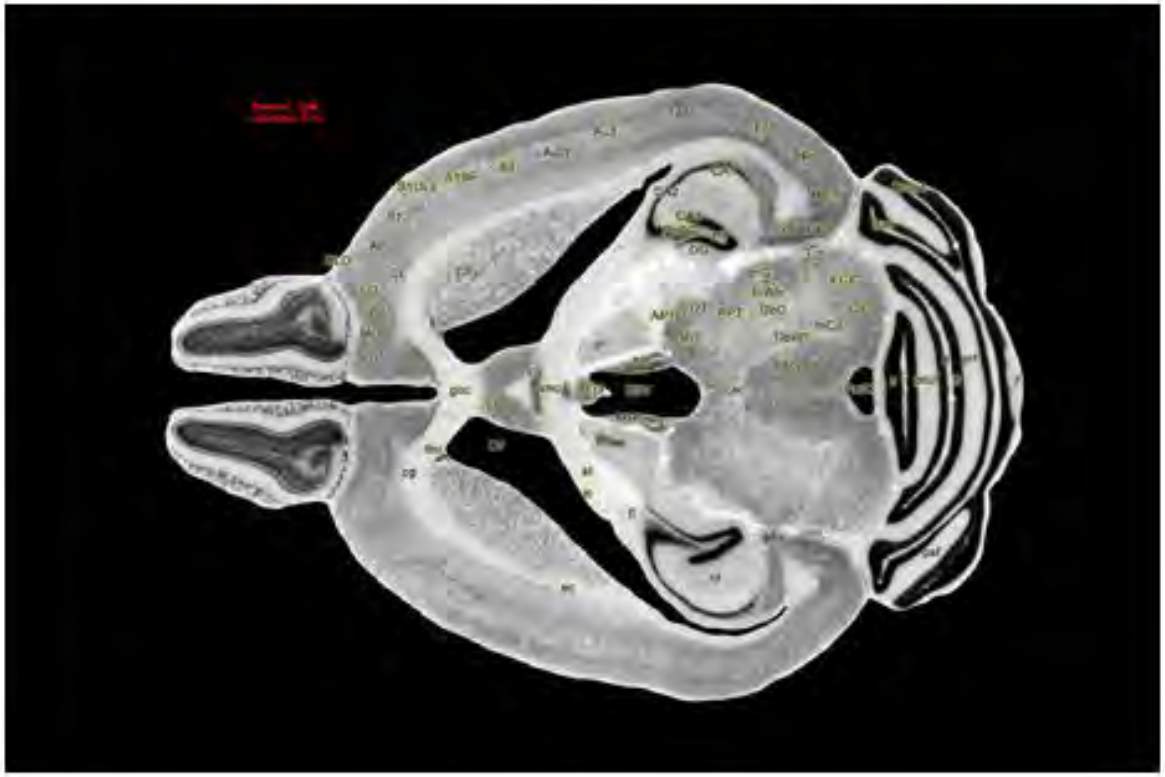


Horizontal sections through the mouse brain

Section 6 horizontal

http://www.mbl.org/

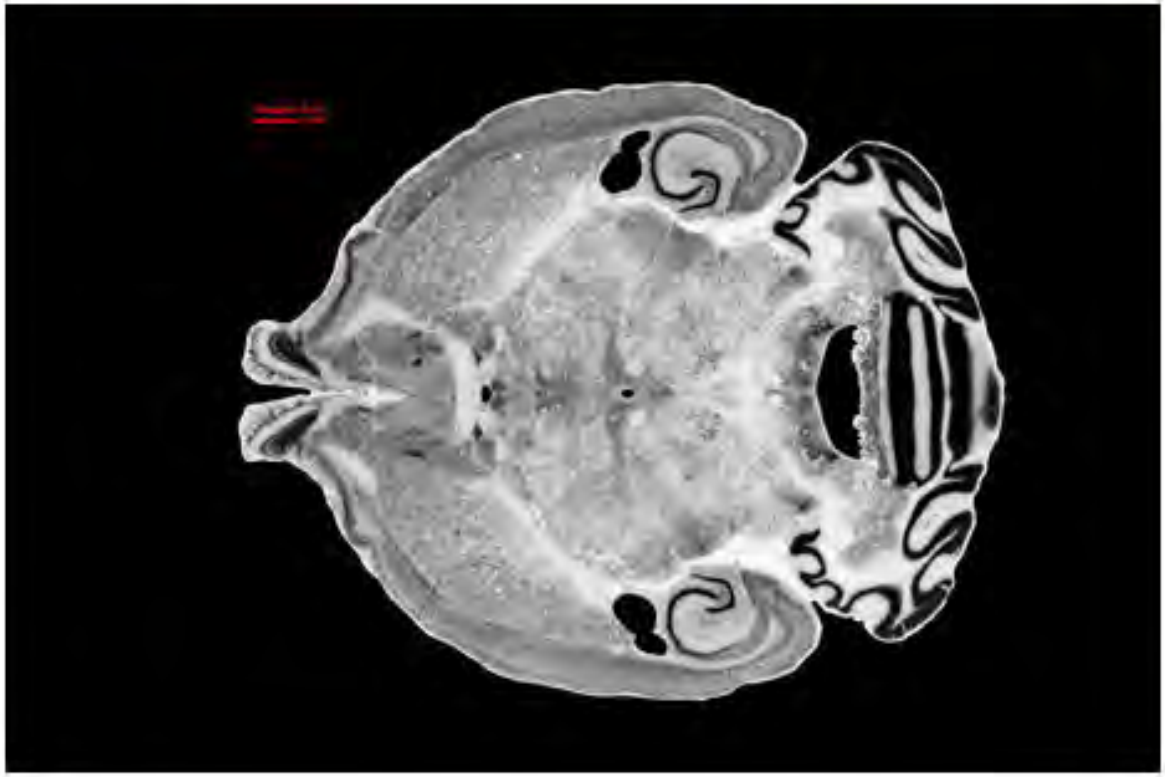
Horizontal sections through the mouse brain

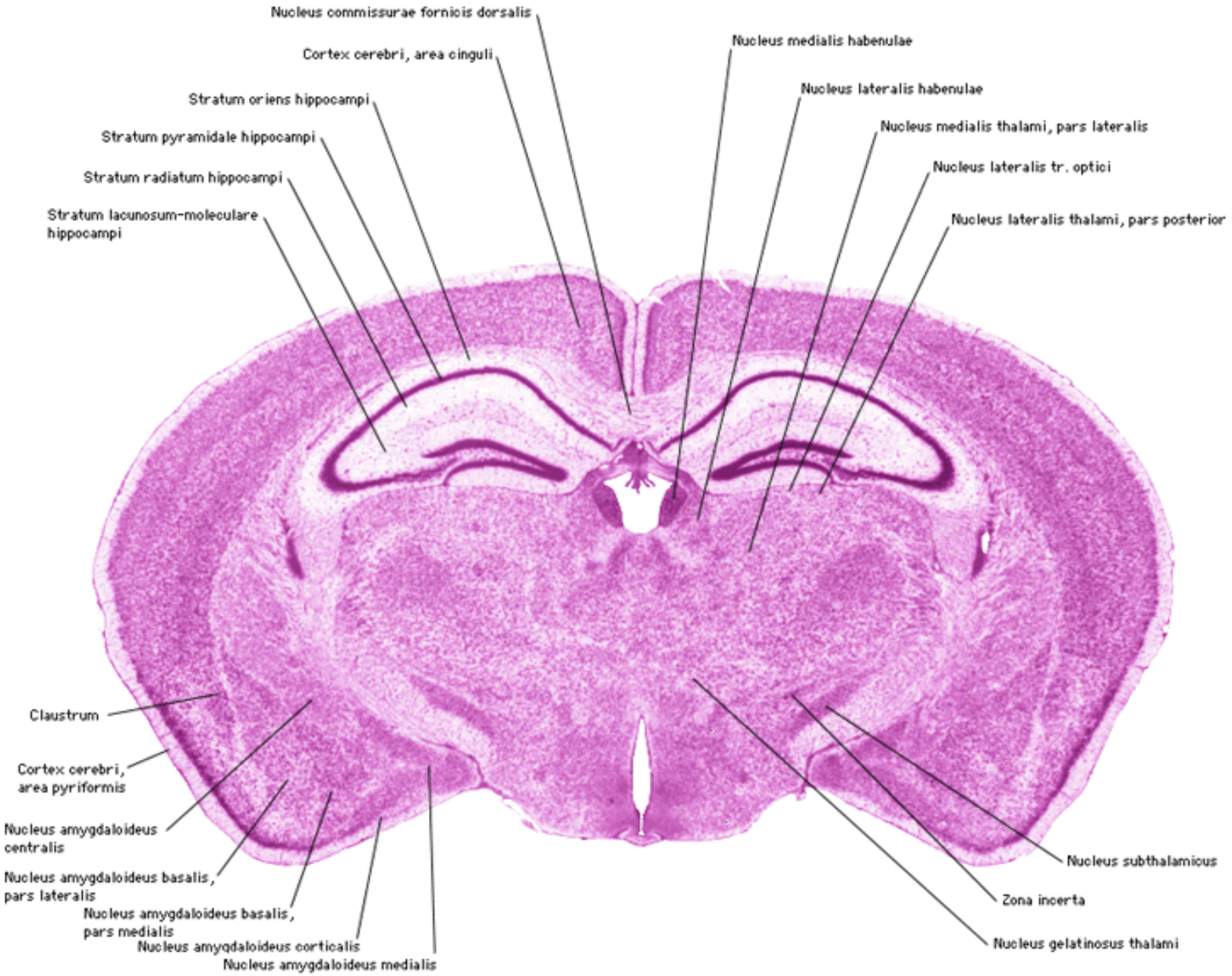


Section 10 horizontal

http://www.mbl.org/

Horizontal sections through the mouse brain




Nucleus commissurae fornicis dorsalis
Cortex cerebri, area cinguli
Stratum oriens hippocampi
Stratum pyramidale hippocampi
Stratum radiatum hippocampi
Stratum lacunosum-moleculare hippocampi
Nucleus medialis habenulae
Nucleus lateralis habenulae
Nucleus medialis thalami, pars lateralis
Nucleus lateralis tr. optici
Nucleus lateralis thalami, pars posterior
Claustrum
Cortex cerebri, area pyriformis
Nucleus amygdaloideus centralis
Nucleus amygdaloideus basalis, pars lateralis
Nucleus amygdaloideus basalis, pars medialis
Nucleus amygdaloideus corticalis
Nucleus amygdaloideus medialis
Nucleus subthalamicus
Zona incerta
Nucleus gelatinosus thalami

Brain Regions

- Olfactory Bulb
- Frontal Pole
- Pyriform Cortex
- Septo-striatal
- Septo-diencephalic
- Rostral Diencephalon
- Caudal Diencephalon
- Rostral Mesencephalon
- Caudal Mesencephalon
- Rostral Cerebellum
- Caudal Cerebellum
- Low Medulla
- Spinal Cord

Nissl

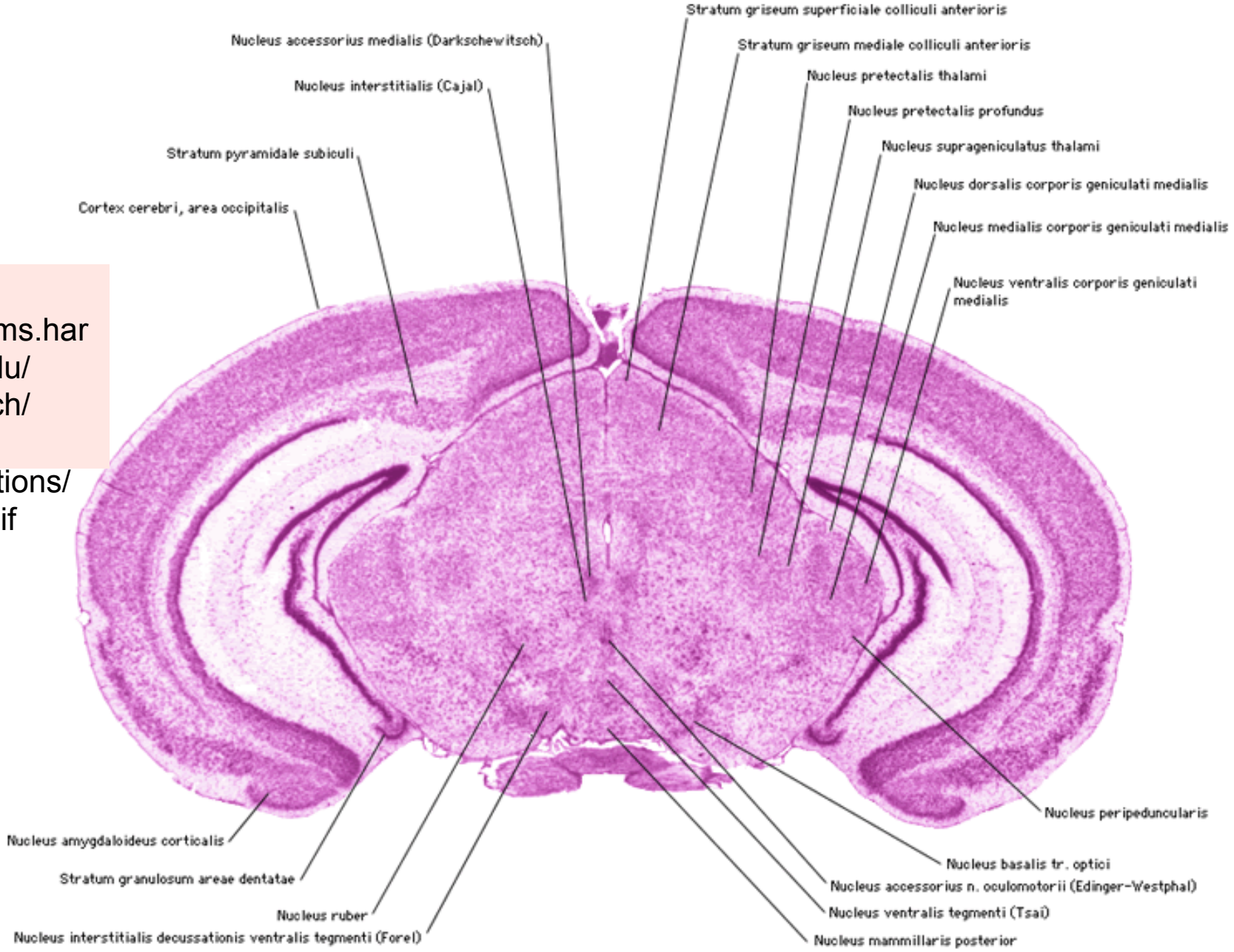
Comp

Myelin

455

Labels

http://www.hms.harvard.edu/research/brain/annotations/355N.gif



What are the cells that are found in the brain?

Grey matter

White matter

NEURONS

SUPPORTING CELLS or GLIA:

--ASTROGLIA or astrocytes

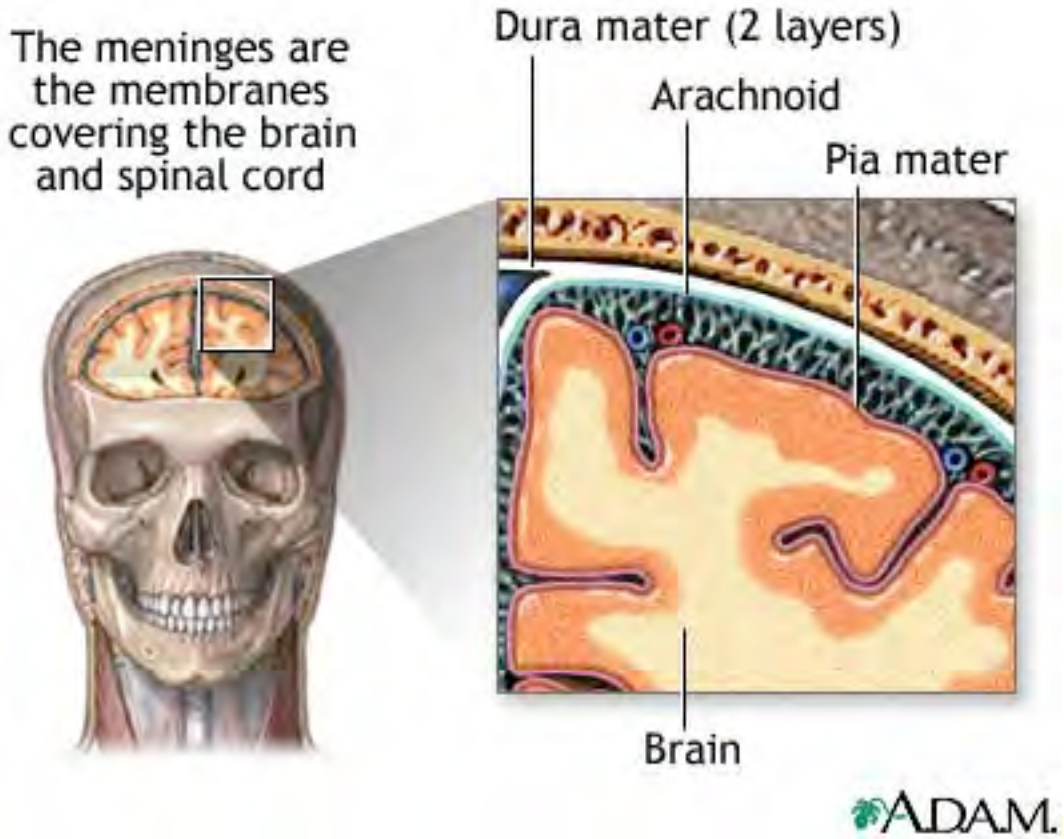
--OLIGODENDROGLIA

--MICROGLIA

BLOOD VESSELS

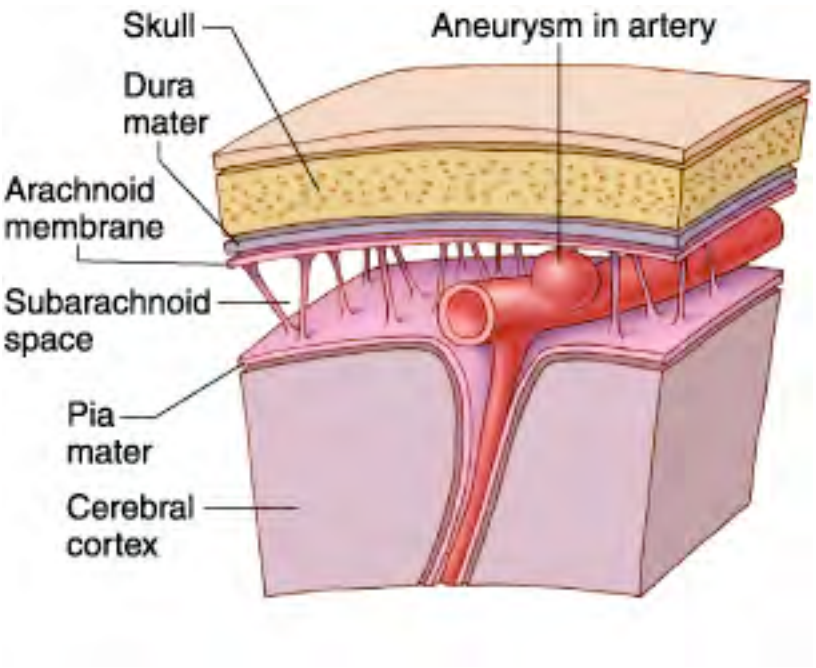
EPENDYMAL CELLS line the ventricles, which make cerebrospinal fluid (CSF)

MENINGEAL CELLS on the surface of the brain



The **meninges** is the system of membranes which envelopes the central nervous system.

The meninges consist of three layers: the dura mater, the arachnoid mater, and the pia mater. The primary function of the meninges and of the cerebrospinal fluid is to protect the central nervous system.



Pathology terms:

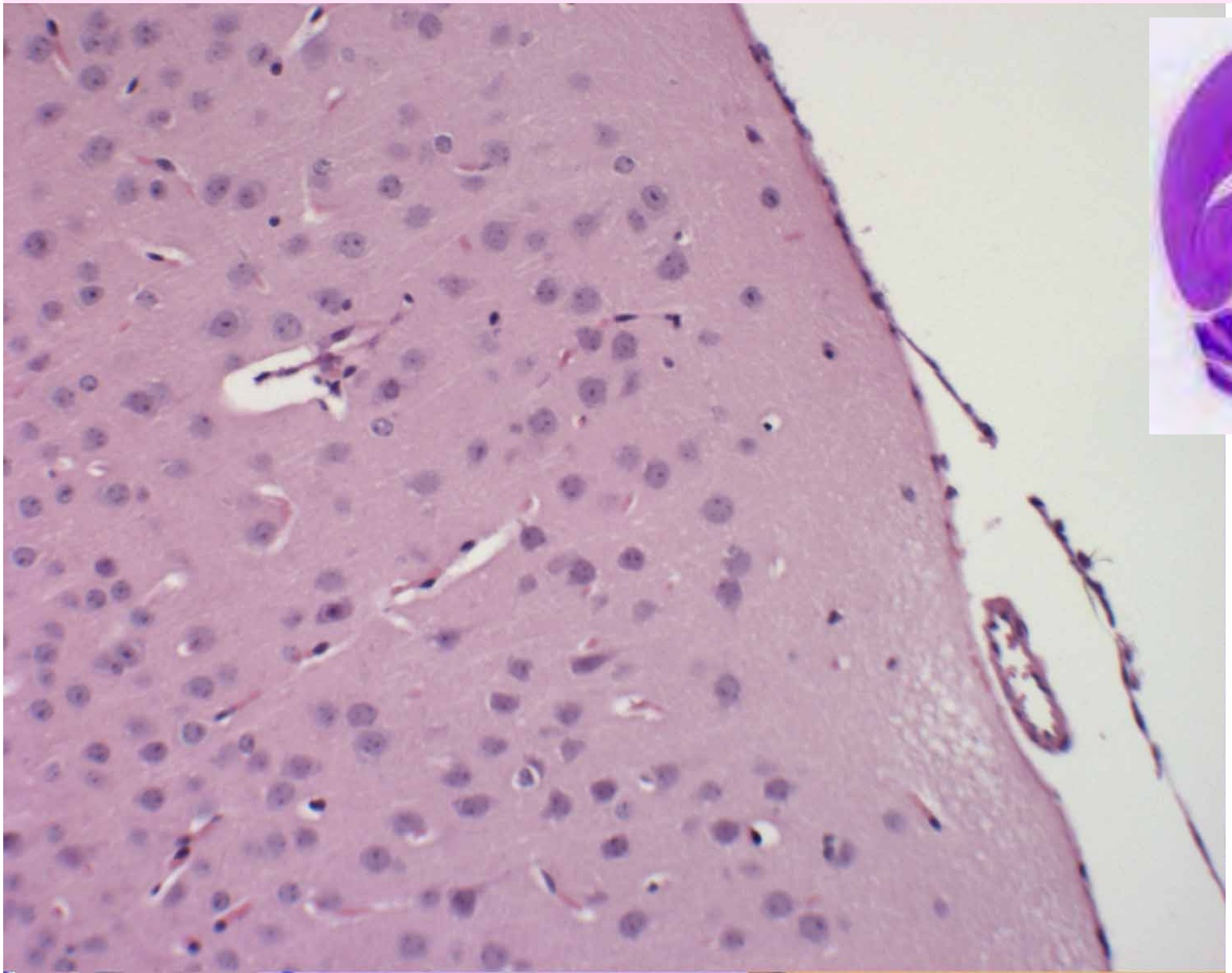
Sub-dural hemorrhage

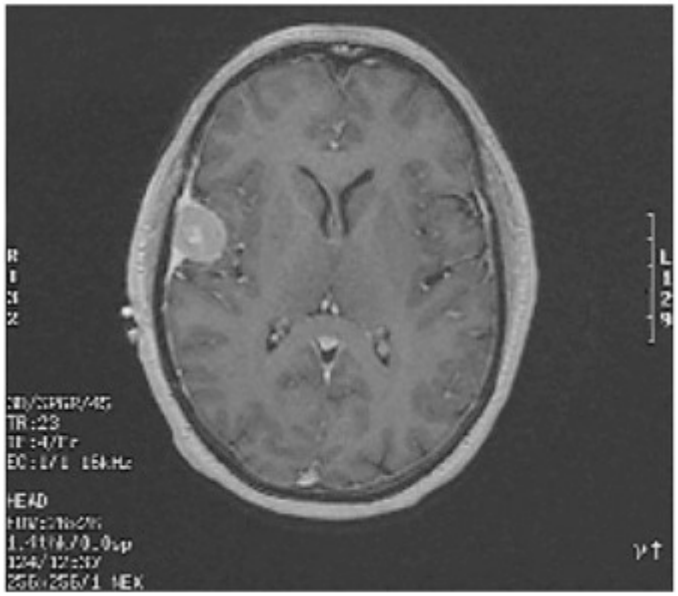
Sub-arachnoid hemorrhage

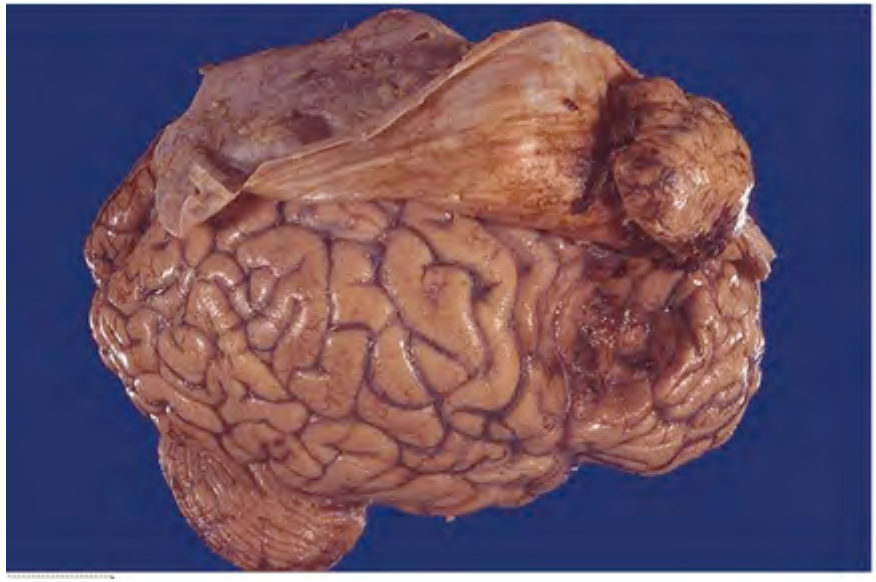
Meningitis

Meningioma

Meninges on the outside of paraffin sections of Mouse Brain with H&E stain

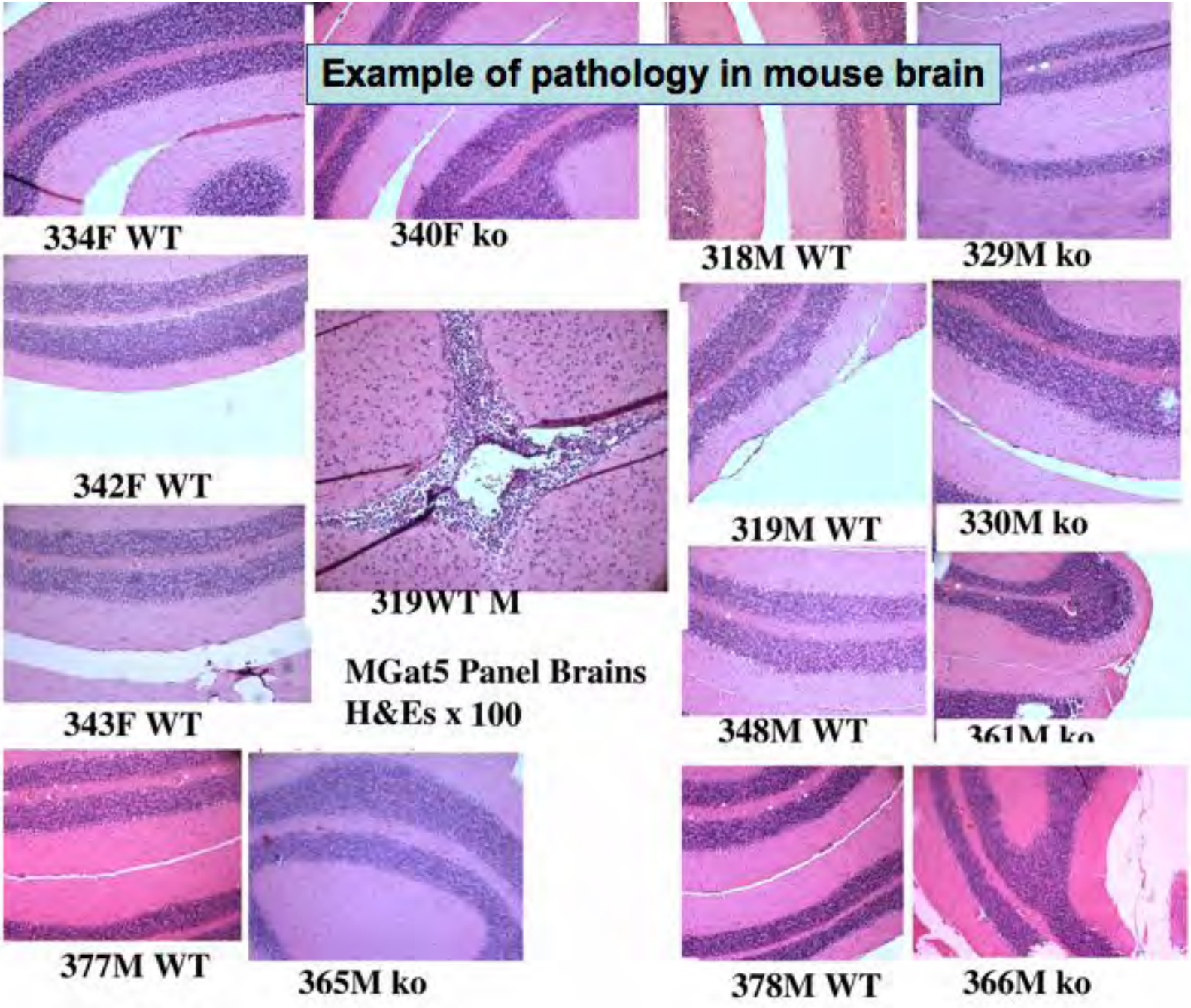






Meningioma MRI (magnetic resonance imaging and gross appearance

http://library.med.utah.edu/WebPath/CNSHTML/CNS116.html


Example of pathology in mouse brain
334F WT
340F ko
318M WT
329M ko
342F WT
319M WT
330M ko
319WT M
343F WT
MGat5 Panel Brains
H&Es x 100
348M WT
361M ko
377M WT
365M ko
378M WT
366M ko

The Ventricles

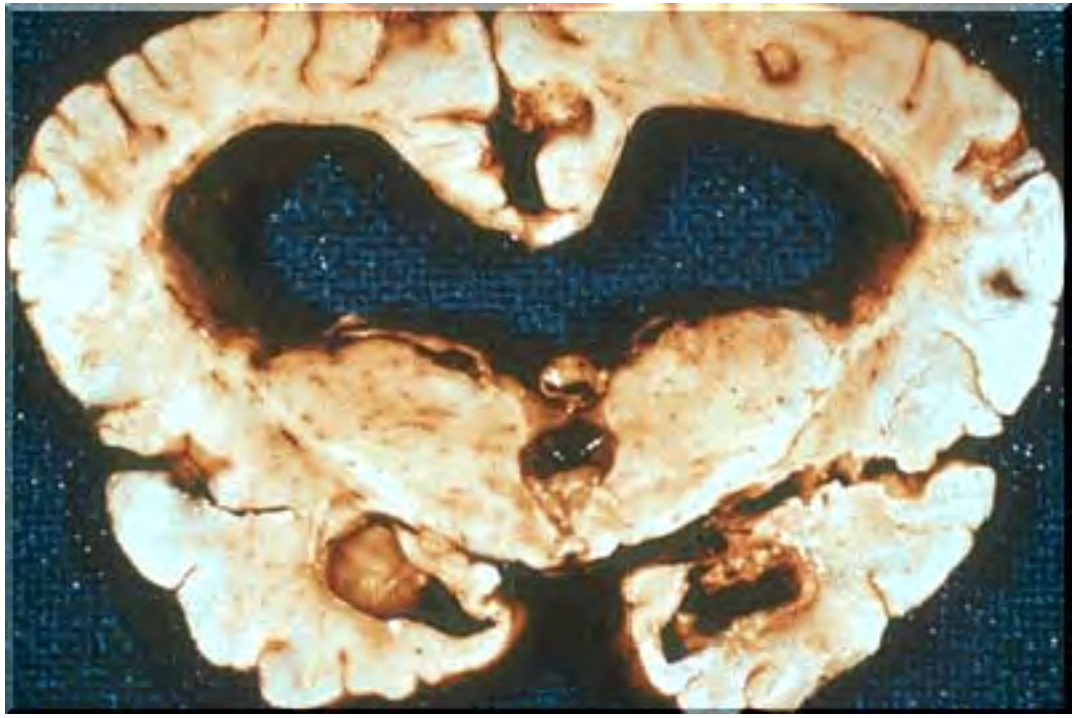
These four spaces are filled with cerebrospinal fluid and protect the brain by cushioning it and supporting its weight.

The two lateral ventricles extend across a large area of the brain. The anterior horns of these structures are located in the frontal lobes. They extend posteriorly into the parietal lobes and their inferior horns are found in the temporal lobes.

The third ventricle lies between the two thalamic bodies. The massa intermedia passes through it and the hypothalamus forms its floor and part of its lateral walls.

The fourth ventricle is located between the cerebellum and the pons.

The four ventricles are connected to one another.



Hydrocephalus: pathologic dilatation of ventricles

Cerebrospinal fluid is a clear liquid produced within spaces in the brain called ventricles. Like saliva it is a filtrate of blood.

It is also found inside the sub-arachnoid space of the meninges which surrounds both the brain and the spinal chord.

In addition, a space inside the spinal cord called the central canal also contains cerebrospinal fluid.

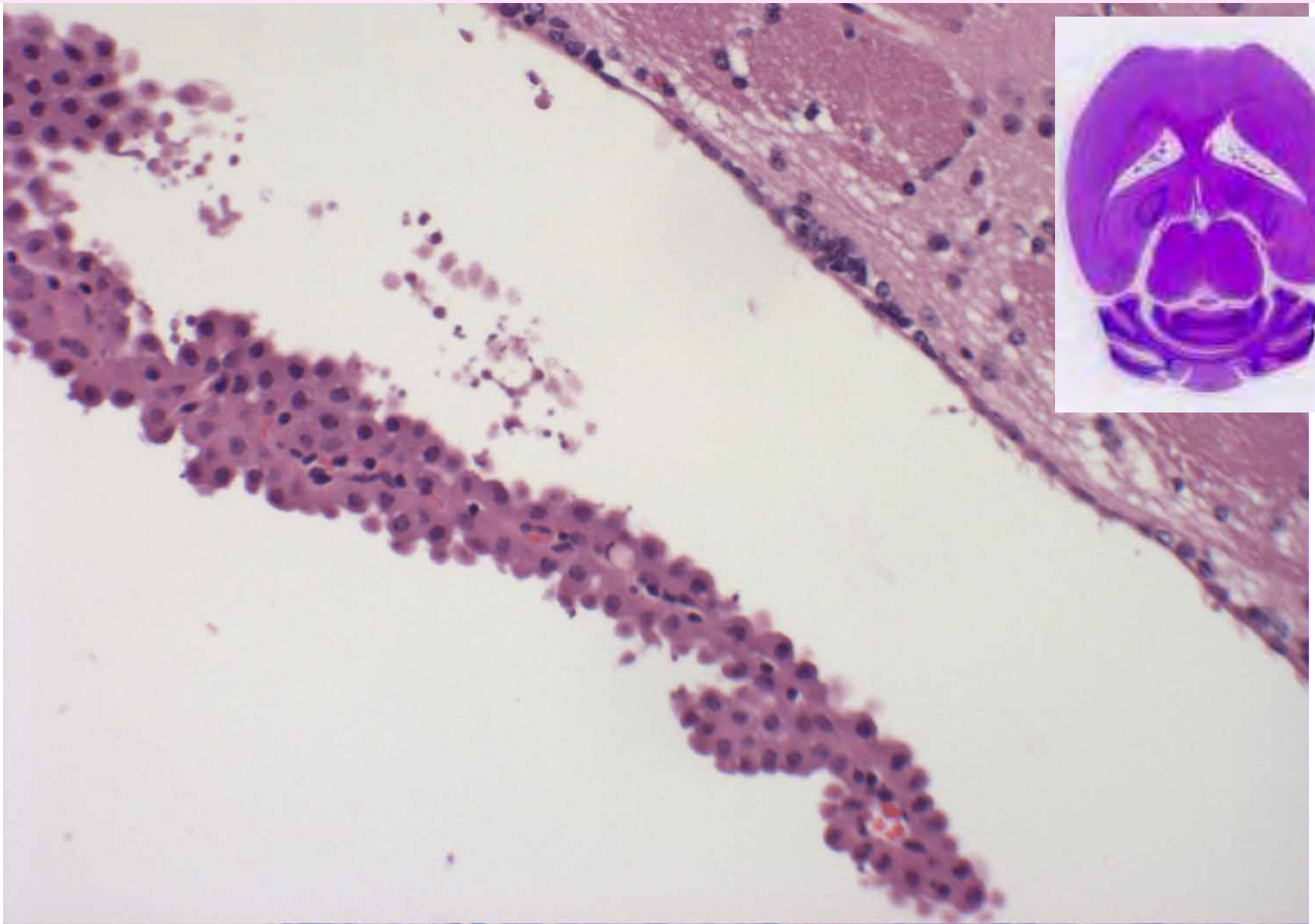
It acts as a cushion for the neuraxis, also bringing nutrients to the brain and spinal cord and removing waste from the system.

Choroid Plexus

All of the ventricles contain choroid plexuses which produce cerebrospinal fluid by allowing certain components of blood to enter the ventricles.

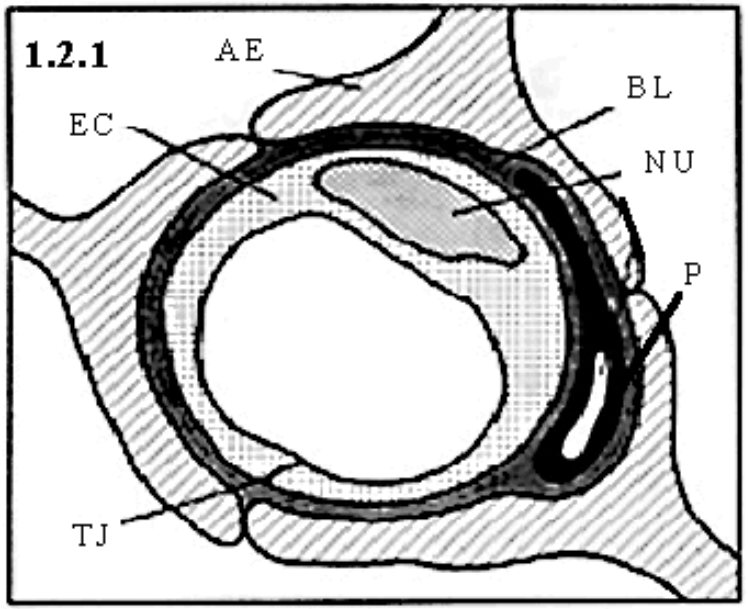
The choroid plexuses are formed by the fusion of the pia mater, the most internal layer of the meninges, and the ependyma, the lining of the ventricles.

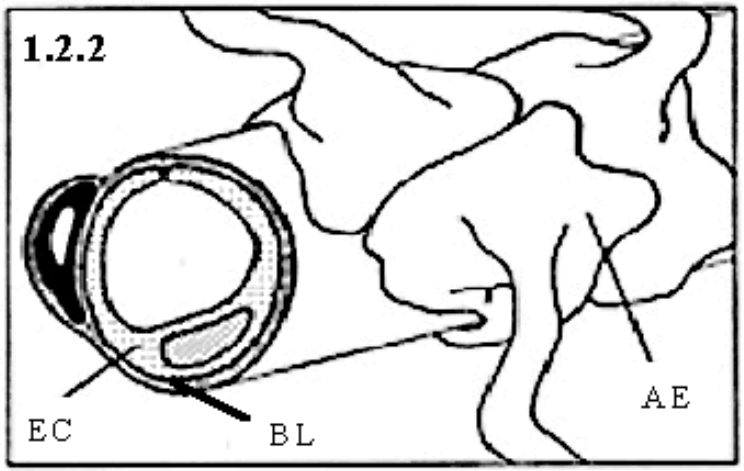
Choroid Plexus in paraffin sections of Mouse Brain with H&E stain



What is the Blood brain barrier?

Over 100 years ago it was discovered that if blue dye was injected into the bloodstream of an animal, that tissues of the whole body EXCEPT the brain and spinal cord would turn blue.





http://users.ahsc.arizona.edu/davis/bbb.htm

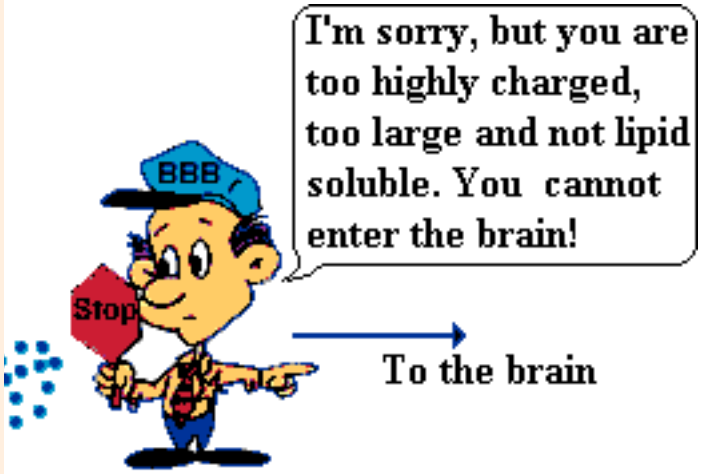
The blood-brain barrier (BBB) is the specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function.

Unlike peripheral capillaries that allow relatively free exchange of substance across / between cells, the BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers.

Thus the BBB is often the rate-limiting factor in determining permeation of therapeutic drugs into the brain.

The blood-brain barrier acts very effectively to protect the brain from many common bacterial infections. Thus, infections of the brain are very rare. However, since antibodies are too large to cross the blood-brain barrier, infections of the brain which do occur are often very serious and difficult to treat.

The blood brain barrier becomes more permeable during inflammation however, meaning some antibiotics can get across. Viruses easily bypass the blood-brain barrier by attaching themselves to circulating immune cells.



http://faculty.washington.edu/chudler/bbb.html

TYPES OF CAPILLARIES

Continuous	No gaps between endothelial cells	Basal Lamina present	Most common
Fenestrated	Endothelial cells separated by gaps of 60-80 nm	Basal Lamina present	Kidney Intestine Endocrine organs
Fenestrated2	Gaps	THICK BASAL lamina	Glomeruli of kidney
Sinusoidal	Gaps	Discontinuous Basal Lamina	Liver Spleen Bone Marrow

Cell Junctions

OCCLUDING: Also known as *tight junctions*

ANCHORING

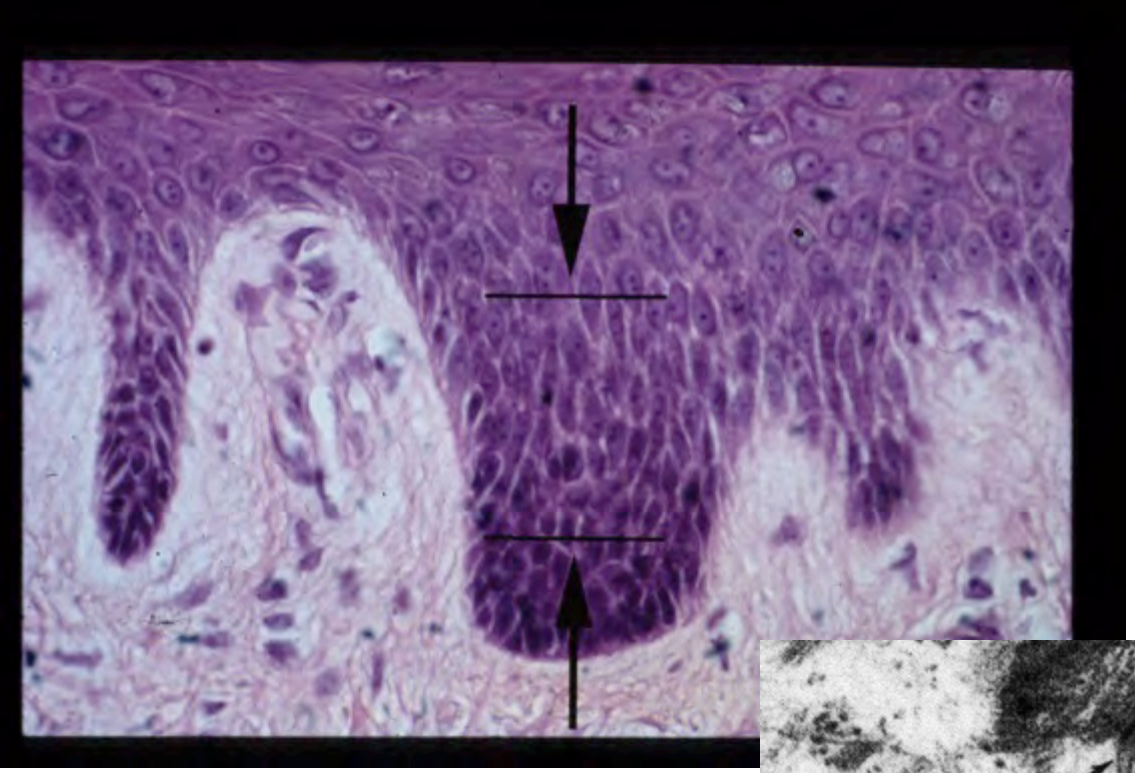
Desmosomes and hemidesmosomes, link with the intermediate filament network

Adherent junctions and focal contacts, link with the actin filament network;

Desmosomes: provide mechanical stability in squamous epithelial cells. Example: E-cadherin

Hemidesmosomes: anchor cells to basement membrane

COMMUNICATING: or Gap junctions: in *Cardiac and Smooth muscle cells*



Functions of the BBB

The BBB has several important functions:

1. **Protects the brain from "foreign substances" in the blood that may injure the brain.**
2. **Protects the brain from hormones and neurotransmitters in the rest of the body.**
3. **Maintains a constant environment for the brain.**

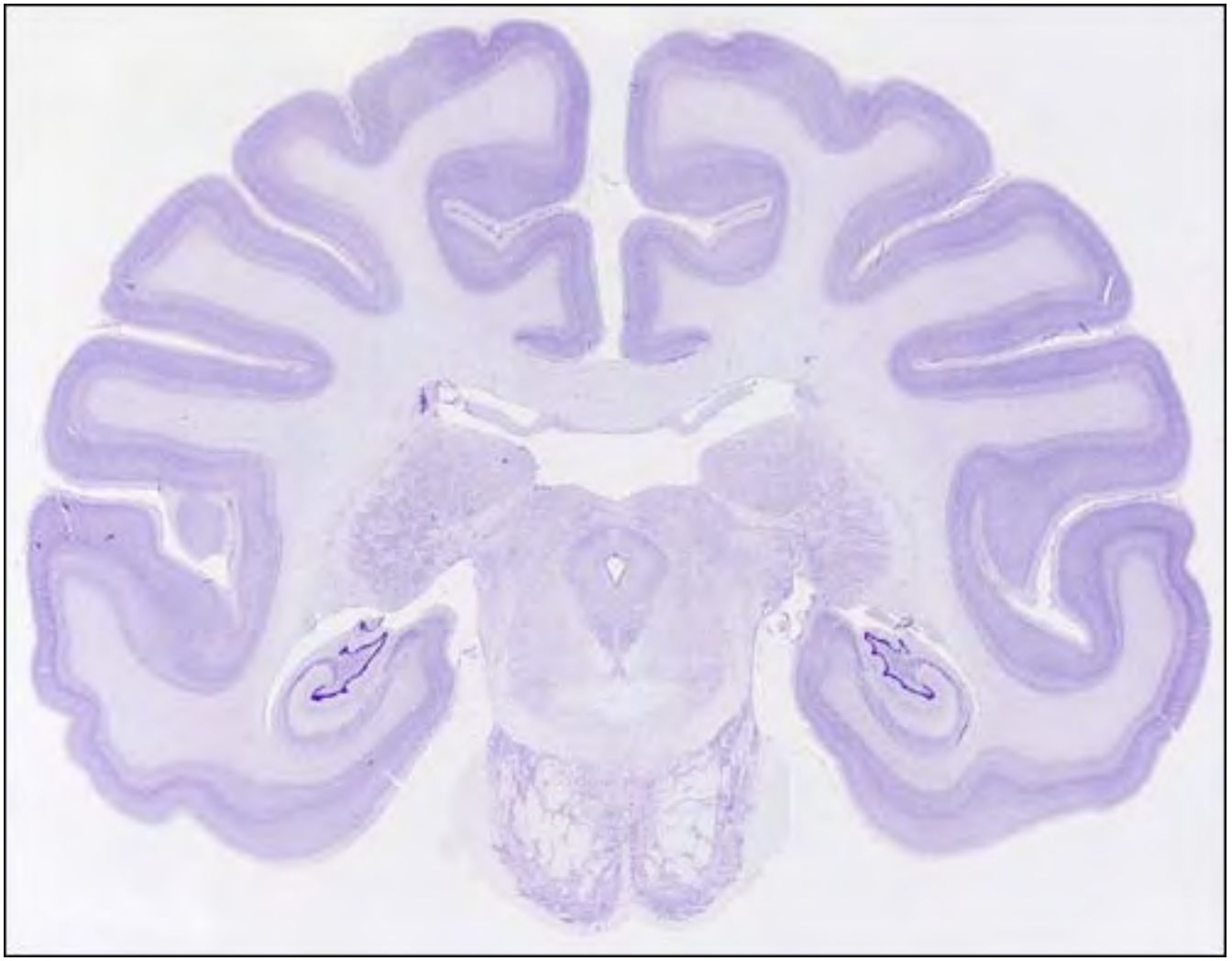
General Properties of the BBB

1. **Large molecules do not pass through the BBB easily.**
2. **Low lipid (fat) soluble molecules do notpenetrate into the brain. However, lipid soluble molecules, such as barbituate drugs, rapidly cross through into the brain.**
3. **Molecules that have a high electrical charge to them are slowed.**

The BBB can be broken down by:

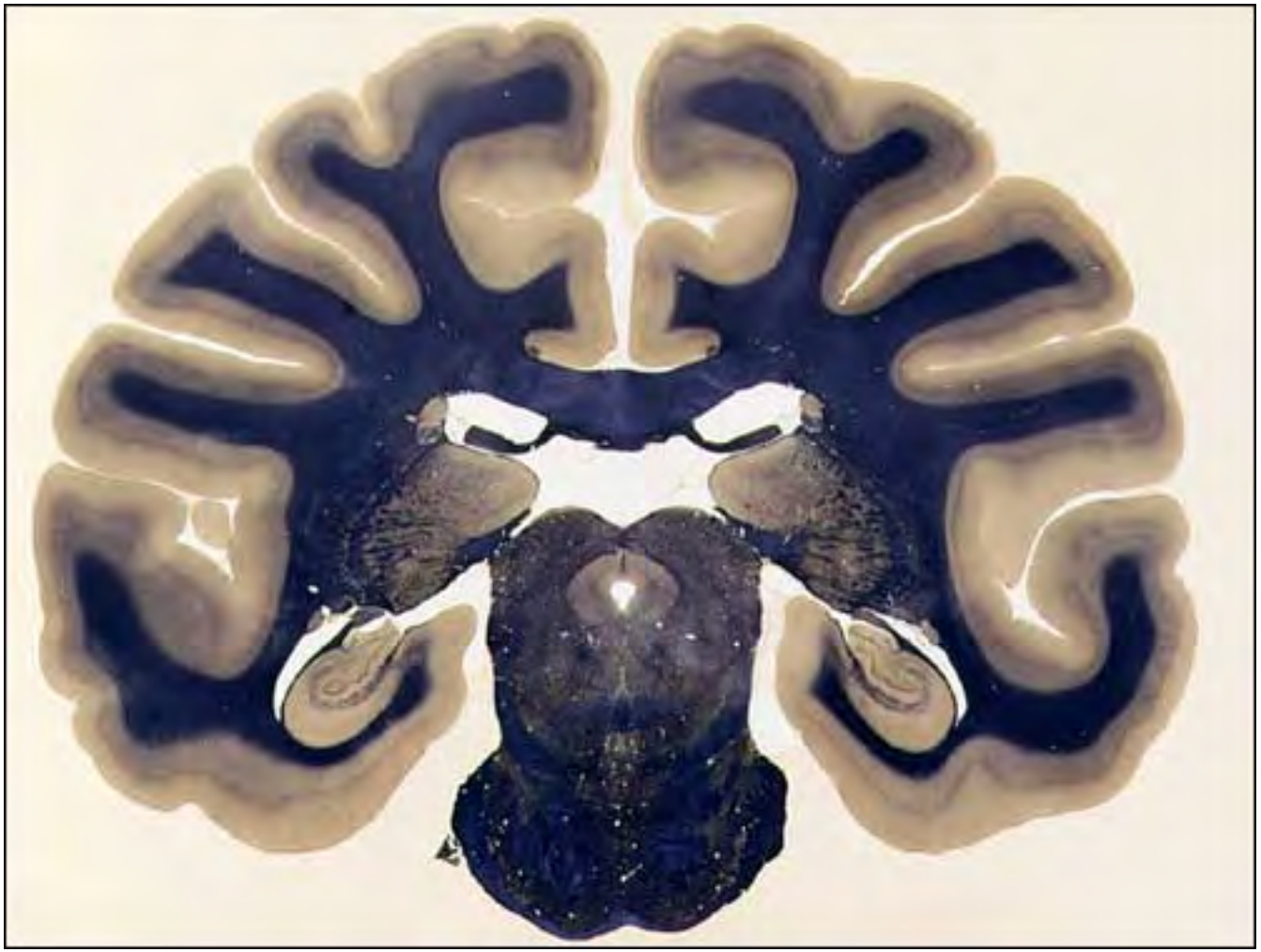
1. **Hypertension (high blood pressure): high blood pressure opens the BBB**
2. **Development: the BBB is not fully formed at birth.**
3. **Hyperosmolitity: a high concentration of a substance in the blood can open the BBB.**
4. **Microwaves: exposure to microwaves can open the BBB.**
5. **Radiation: exposure to radiation can open the BBB.**
6. **Infection: exposure to infectious agents can open the BBB.**
7. **Trauma, Ischemia, Inflammation, Pressure: injury to the brain can open the BBB**

http://faculty.washington.edu/chudler/introb.html



Nissl stain (e.g., cresyl violet, thionin, azure) stains nuclei acids (DNA and RNA). This stain is useful for viewing cell sizes and numbers.

http://www.neuroscienceassociates.com/Stains/weil_myelin.htm



Myelin stain of Human brain

Markers for CELL TYPES FOUND IN THE BRAIN AND SPINAL CORD

--NEURONS (NeuN)

--GLIA: **3 types of supporting GLIA:**

Astrocytes: **the principle cells that respond in a non-specific way to injuries of the nervous system. --marker: GFAP**

Oligodendroglia: **major function: to produce myelin. marker: MBP**

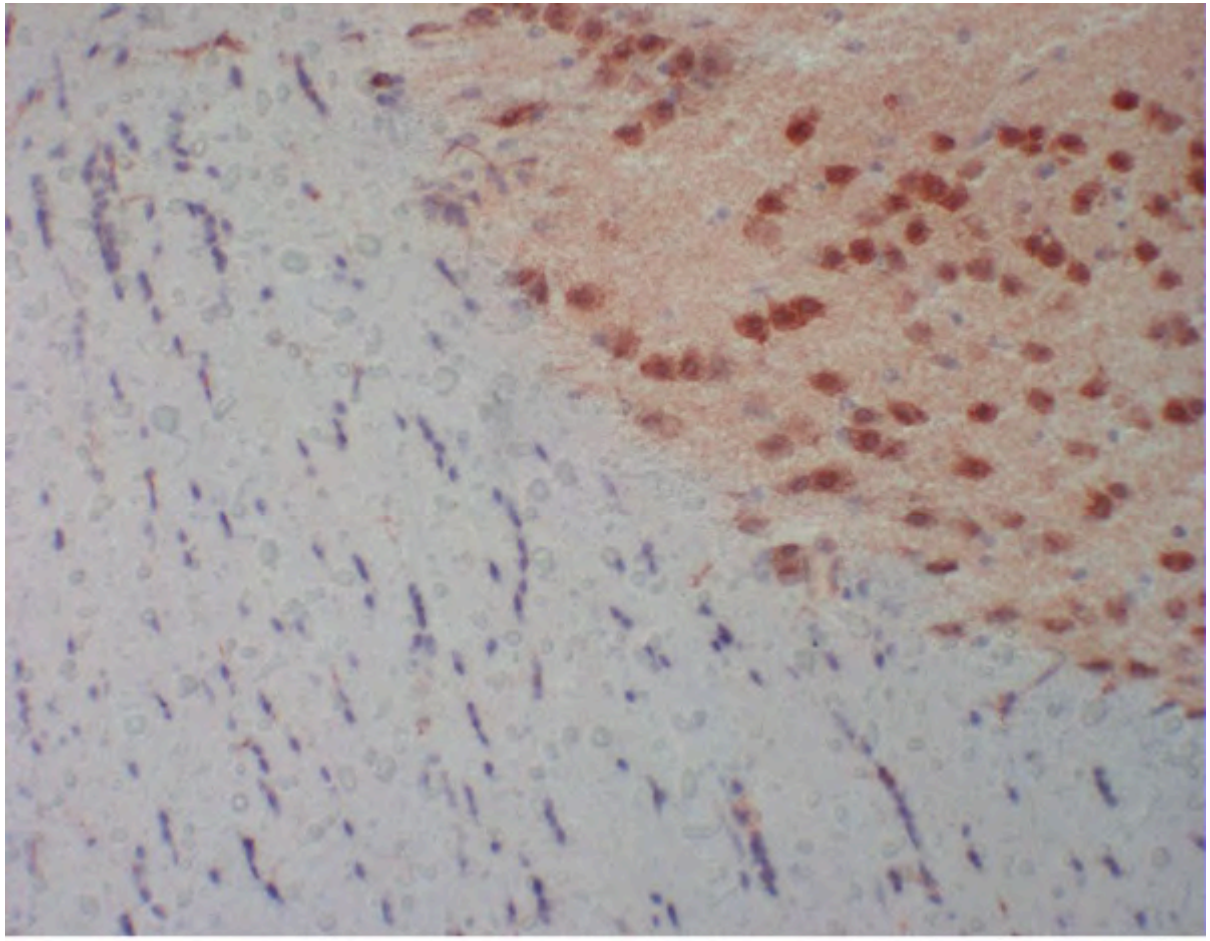
Microglia: **are members of the mononuclear phagocyte system. Marker: CD68**

--EPENDYMAL cells--- lining the ventricles and the choroid plexus, (which makes CSF)

--MENINGEAL cells ---on the outside of the brain

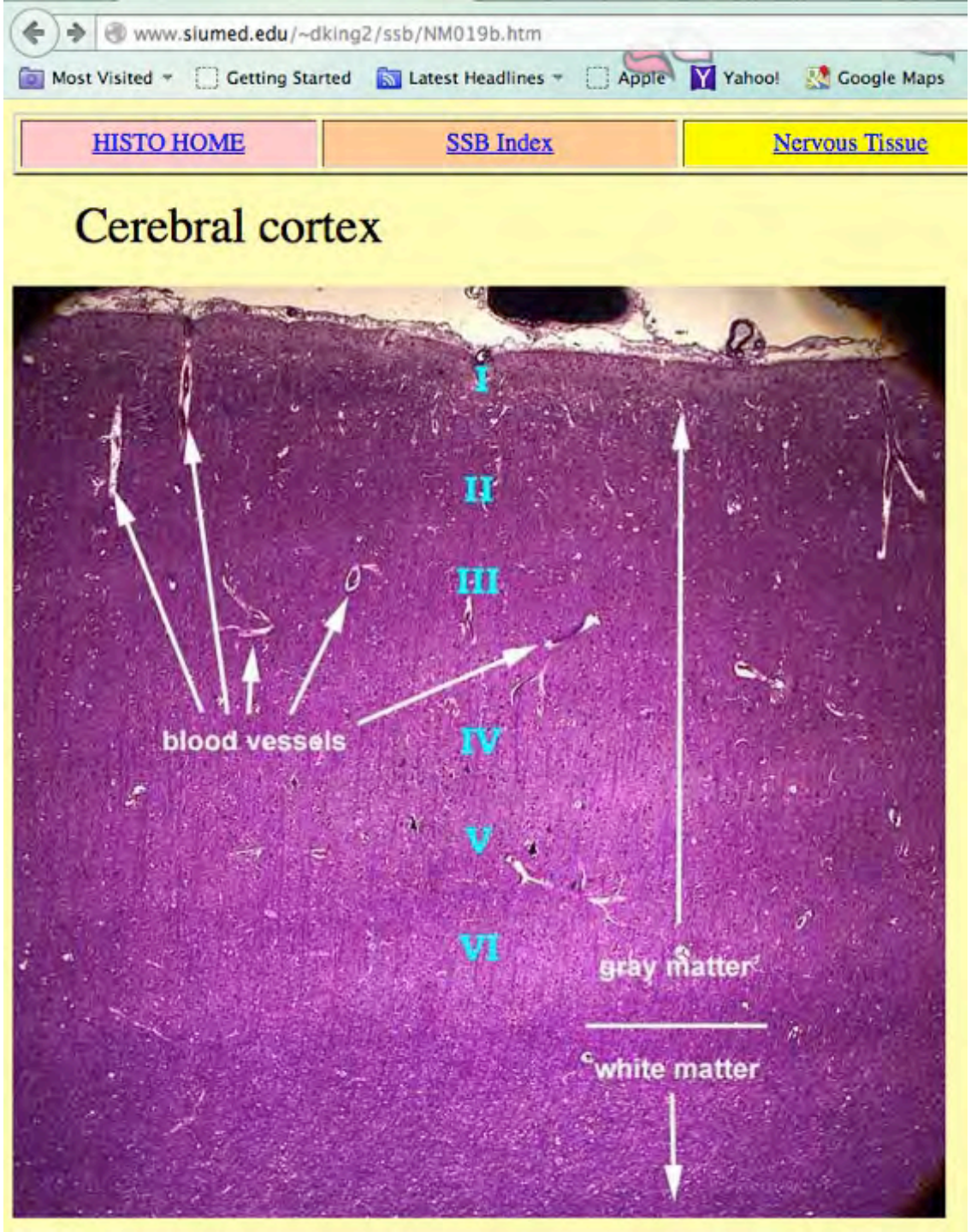
--ENDOTHELIAL cells (CD31)

--SCHWANN cells--peripheral nervous system



Neuronal nuclei identified using anti-NeuN (brown)

Arbitrary layers of the cerebral cortex



Yale Systems Cell Biology

Neurons in the Brain

In the brain, the positions of the gray and white matter are the reverse of what they are in the spinal cord - the gray matter containing cell bodies is external, and the white matter containing nerve fibers is internal. The gray matter of the **cerebral cortex** is divided into 6 layers. The characteristic cell type of the cortex is the **pyramidal cell**, so-called because of their triangular shape. Pyramidal cells have a thick, branching dendrite located at the apex and a long axon that extends toward the white matter.

The **cerebellar cortex** has three layers: an outer **molecular layer** with nerve cell processes, a layer of **Purkinje cells**, and an inner **granular layer** with several other types of neurons. Purkinje cells are very large neurons that possess a tree of branching dendrites that extend into the molecular layer.

Glial Cell in the Central Nervous System

Neuroglia are the main non-nervous cells of the central nervous system. The are present in the extracellular space of nervous tissue, or **neuropil**. You will observe four types of CNS neuroglia in this lab:

- **Astrocytes** are derived from the ectoderm. They are supporting cells that possess many processes and are interposed between neurons, except at the site of synapses. These cells regulate the metabolic environment of the extracellular space and are important for scarring during traumatic injury to the brain. Astrocytes occur as two histological types. **Protoplasmic astrocytes** have broad, symmetrical processes and are usually confined to the gray matter, whereas **fibrous astrocytes** have asymmetrical processes and are typically confined to white matter.
- **Ependymal cells** line the ventricles of the brain and the central canal of the spinal cord. These are derived from epithelial cells and produce cerebrospinal fluid.
- **Oligodendrocytes** are derived from the ectoderm and are the myelinating cells of the central nervous system.
- **Microglia** are of mesodermal origin and are situated among neurons and around capillaries. These cells are phagocytic and are the CNS counterpart of connective tissue macrophages (also of mesodermal origin).

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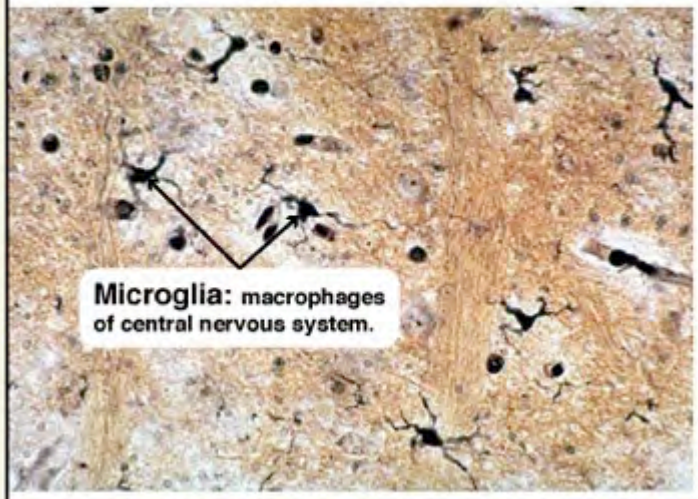
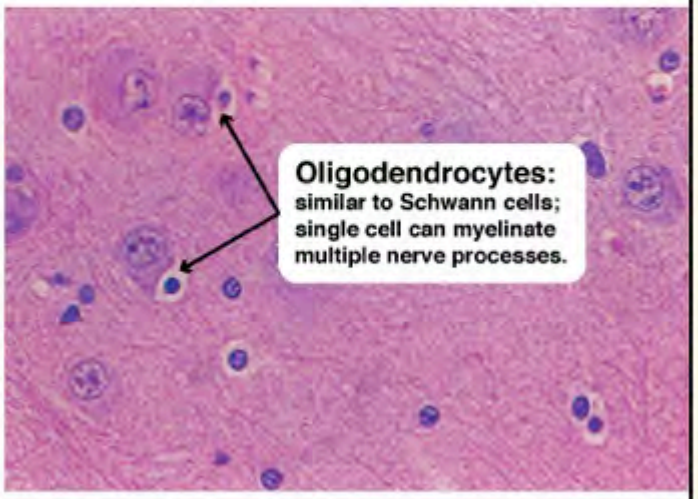
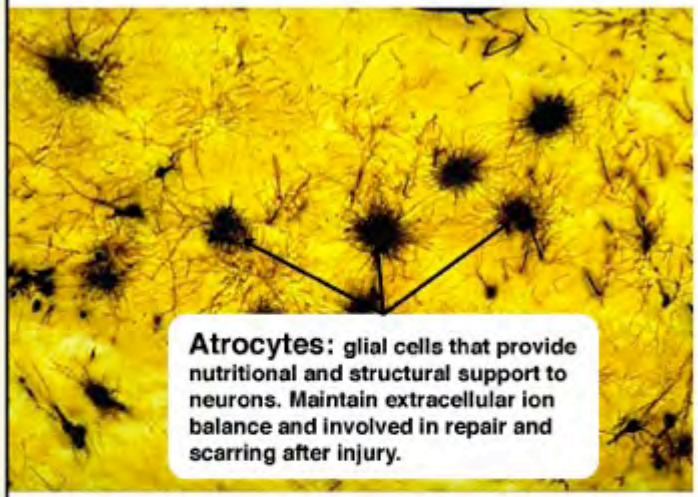
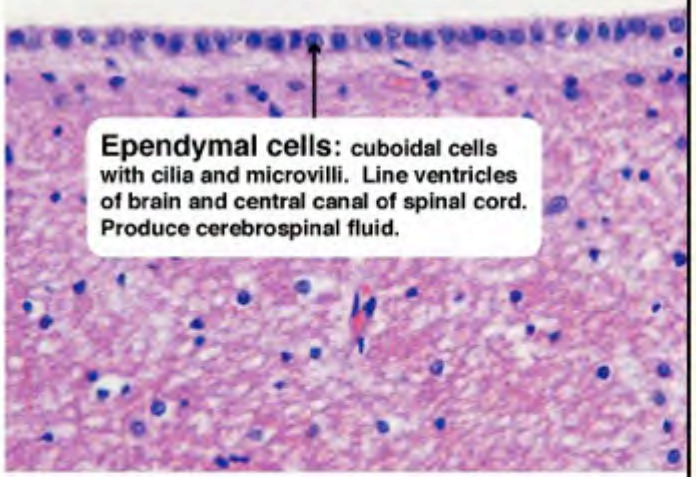
Neuroglia





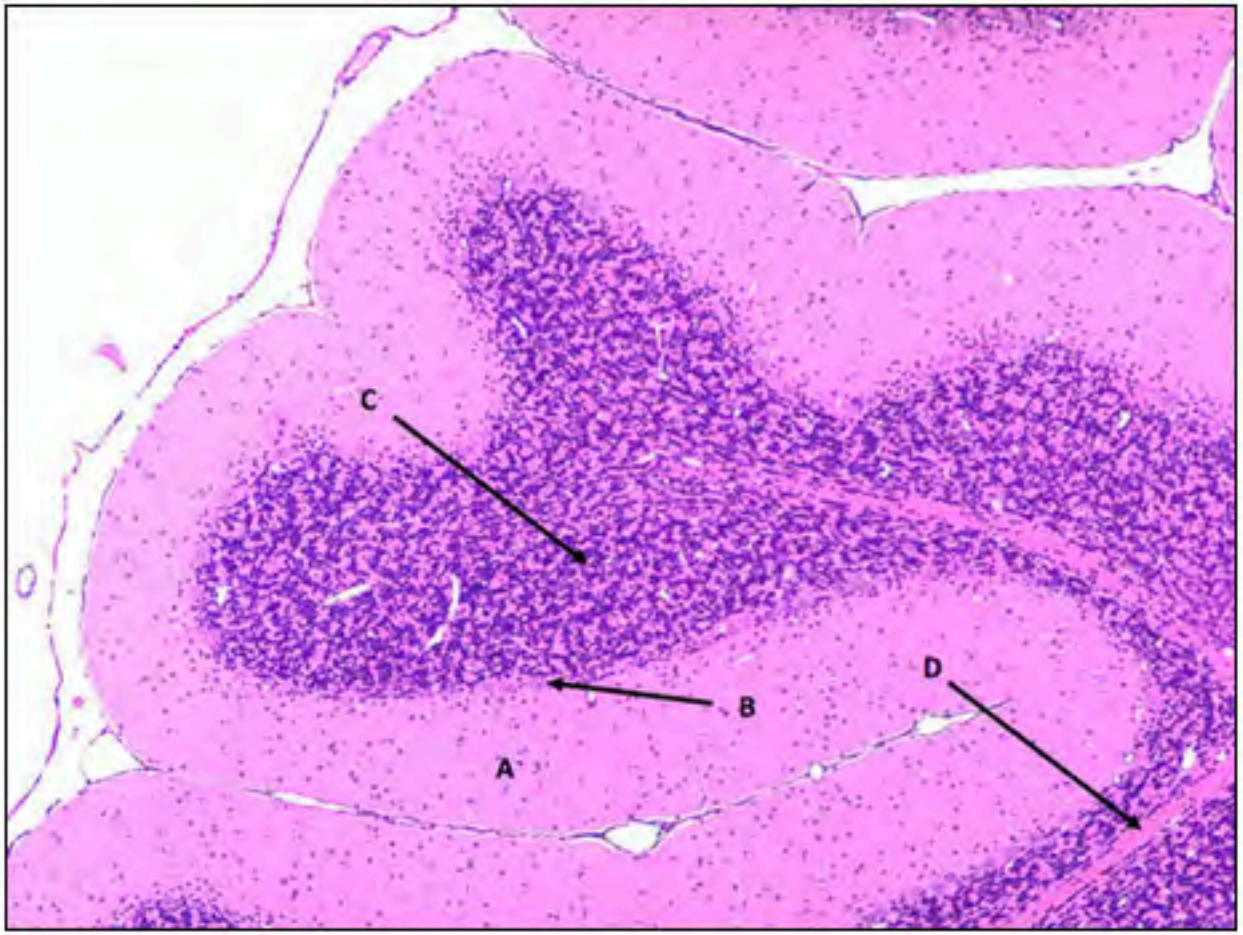




The neuroglia are the non-neuronal cells of the central nervous system and play a crucial role in the development and maintenance of the neurons that they support.

Show Labels Hide Labels

Cerebellum histology



Answer: *A= molecular layer, B = Purkinje layer, C = granular layer, D = white matter*

Spinal Cord

medcell.med.yale.edu/systems_cell_biology_old/nervous/spinal_cord.php

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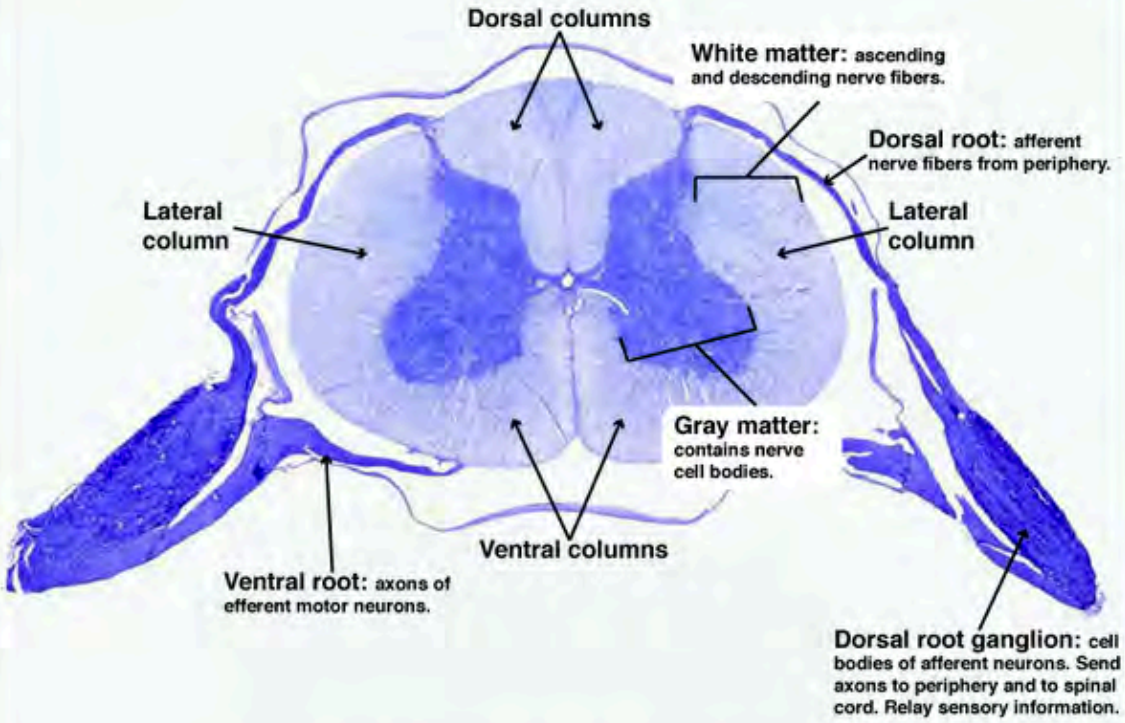
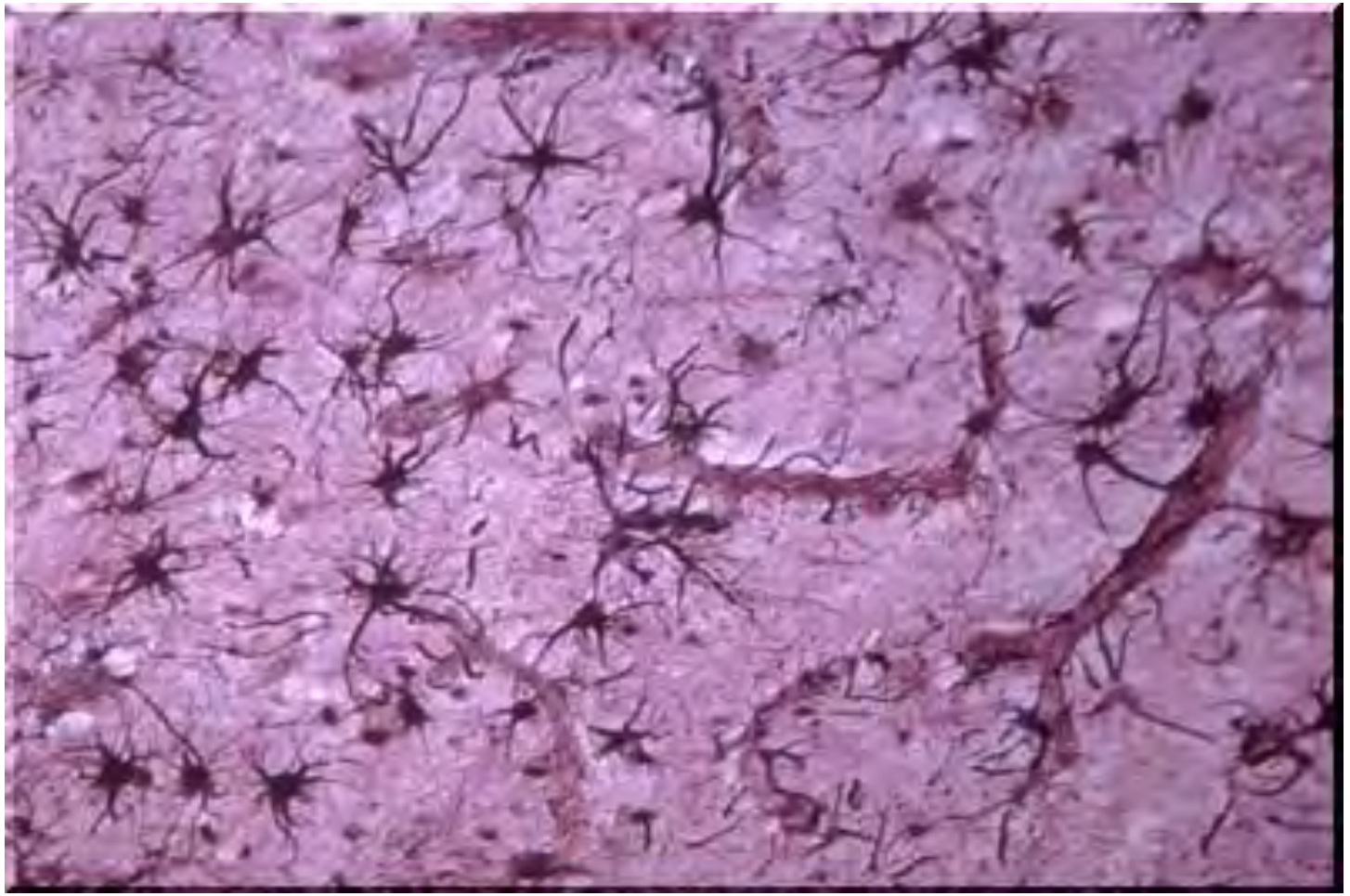
Spinal Cord



Many important features of the spinal cord are visible in this cross section. The central canal lies in the center of the cord and contains cerebrospinal fluid. It is lined by ependymal cells, a type of neuroglia that produces CSF.

What classes of neurons have their cell bodies in the dorsal horn and ventral horn? What is the direction of the action potentials for both of these classes?

Show Labels | Hide Labels



Astrocytes identified using anti-GFAP (glial fibrillary acidic protein)

Astrocytoma – benign tumor

Glioblastoma multiforme--malignant

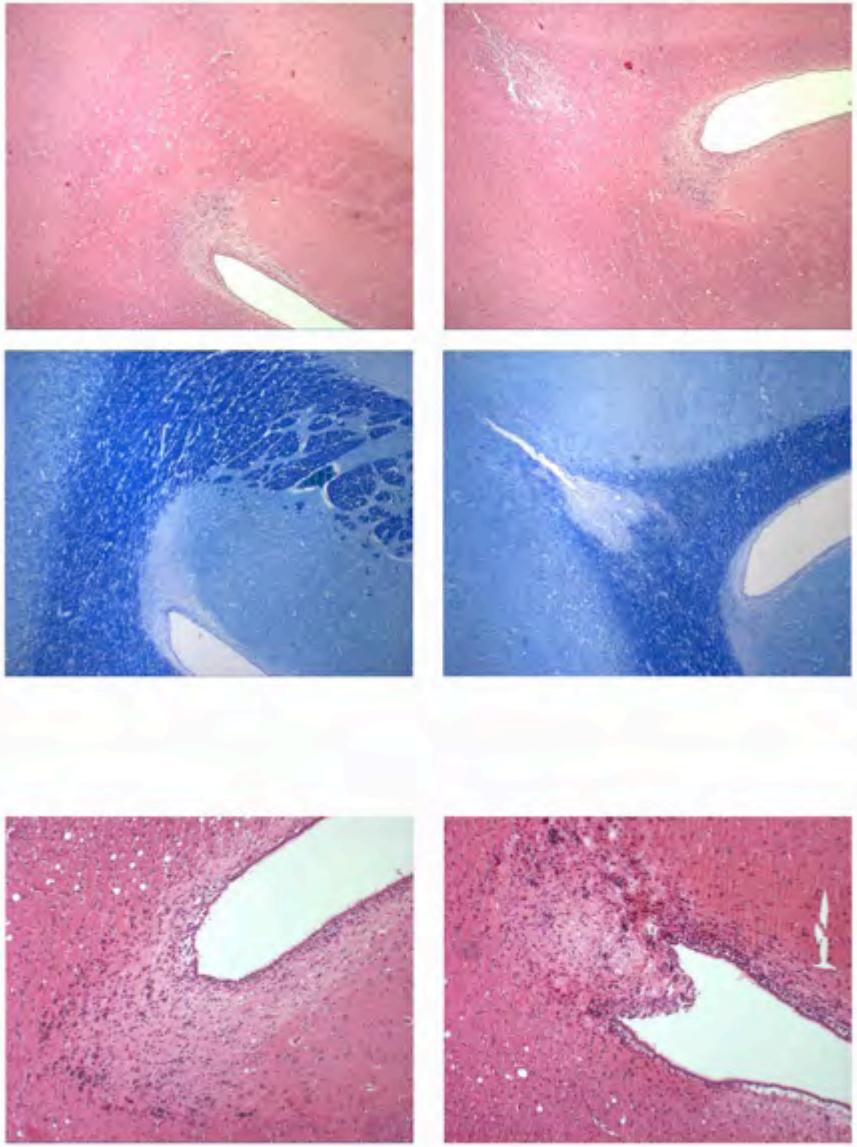
Cells that are responsible for Myelin production

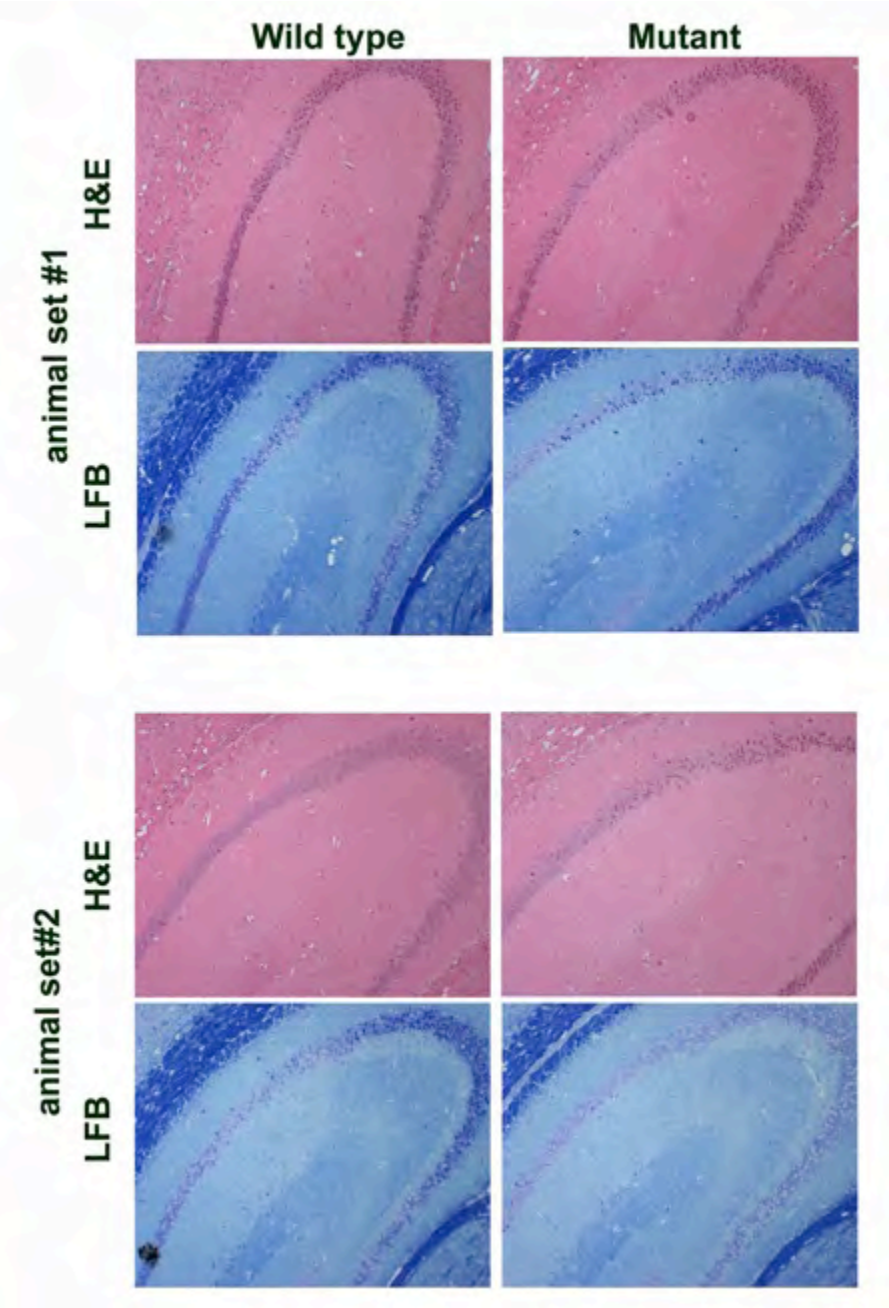
Oligodendroglia: in the central nervous system

Schwann cells: in the peripheral nervous system

Histochemical stain: Luxol Fast Blue (LFB)

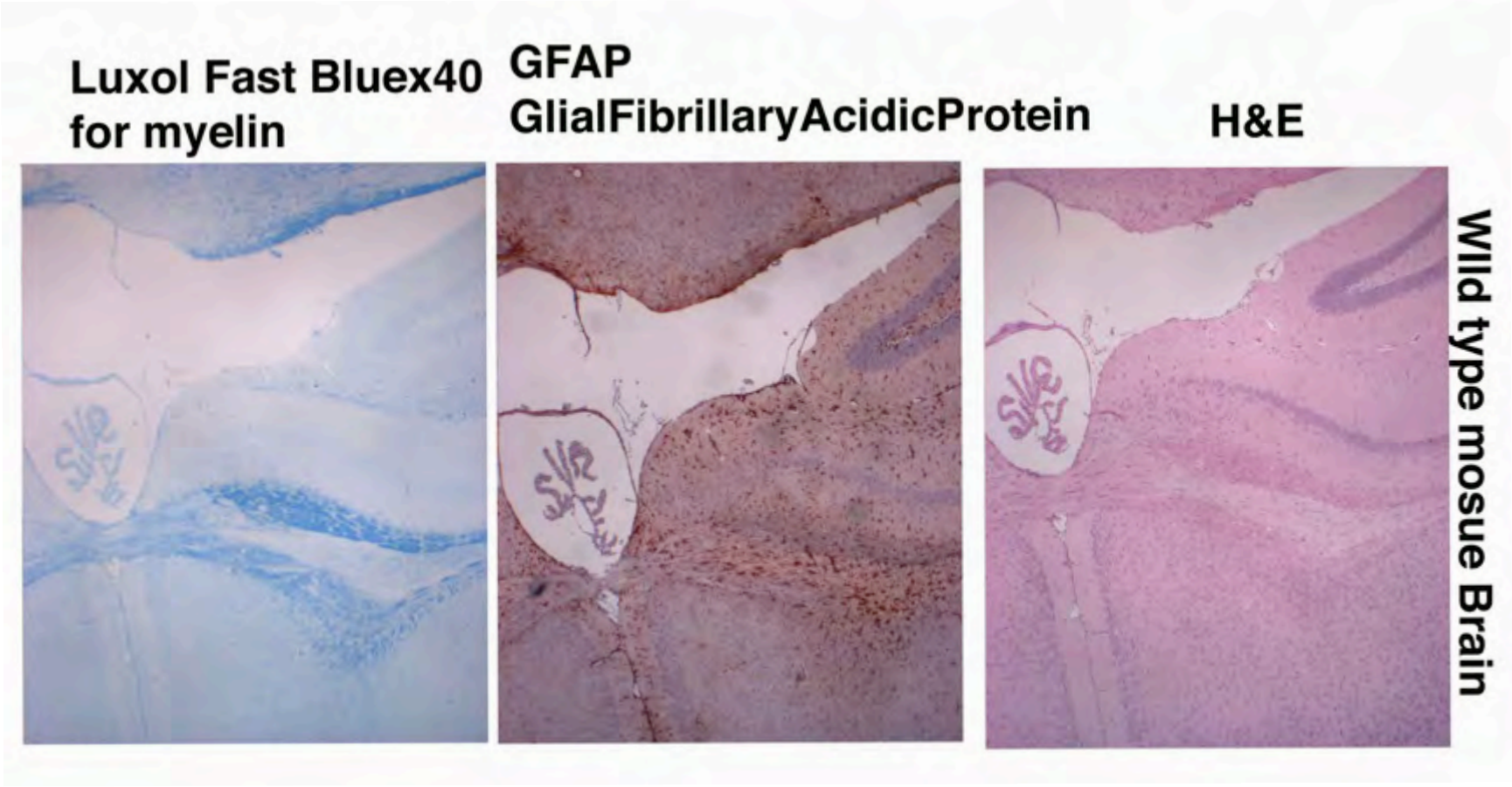
Immunohistochemistry using markers for Myelin Basic Protein (MBP)





Abnormality seen on nuclei in the mutant hippocampus after LFB was followed with nuclear crystal violet stain

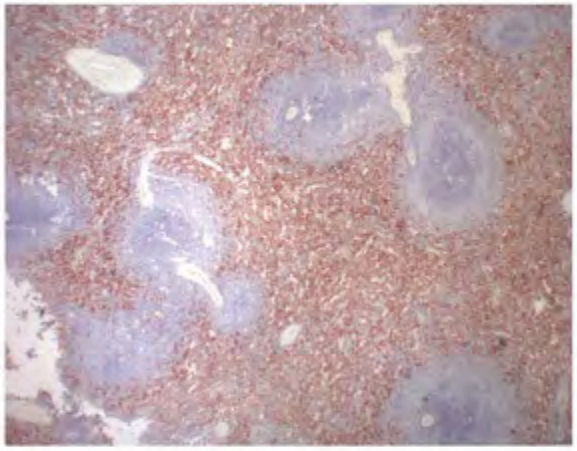
Comparison of different stains on mouse brain sections



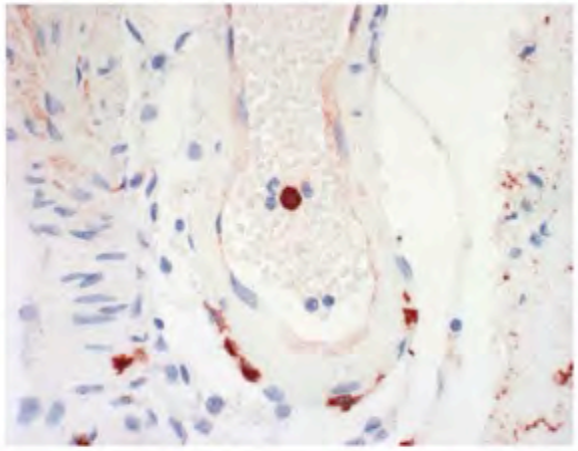
Microglia: macrophages in the brain

Rabbit anti-Iba-1 on paraffin sections

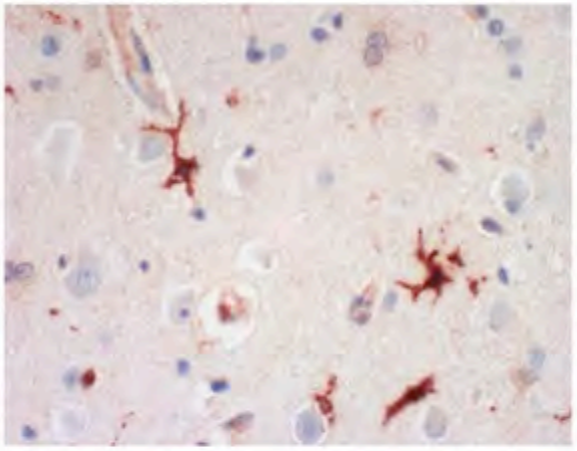
Human Spleen x40



Human Spleen x400



Human Brain x400



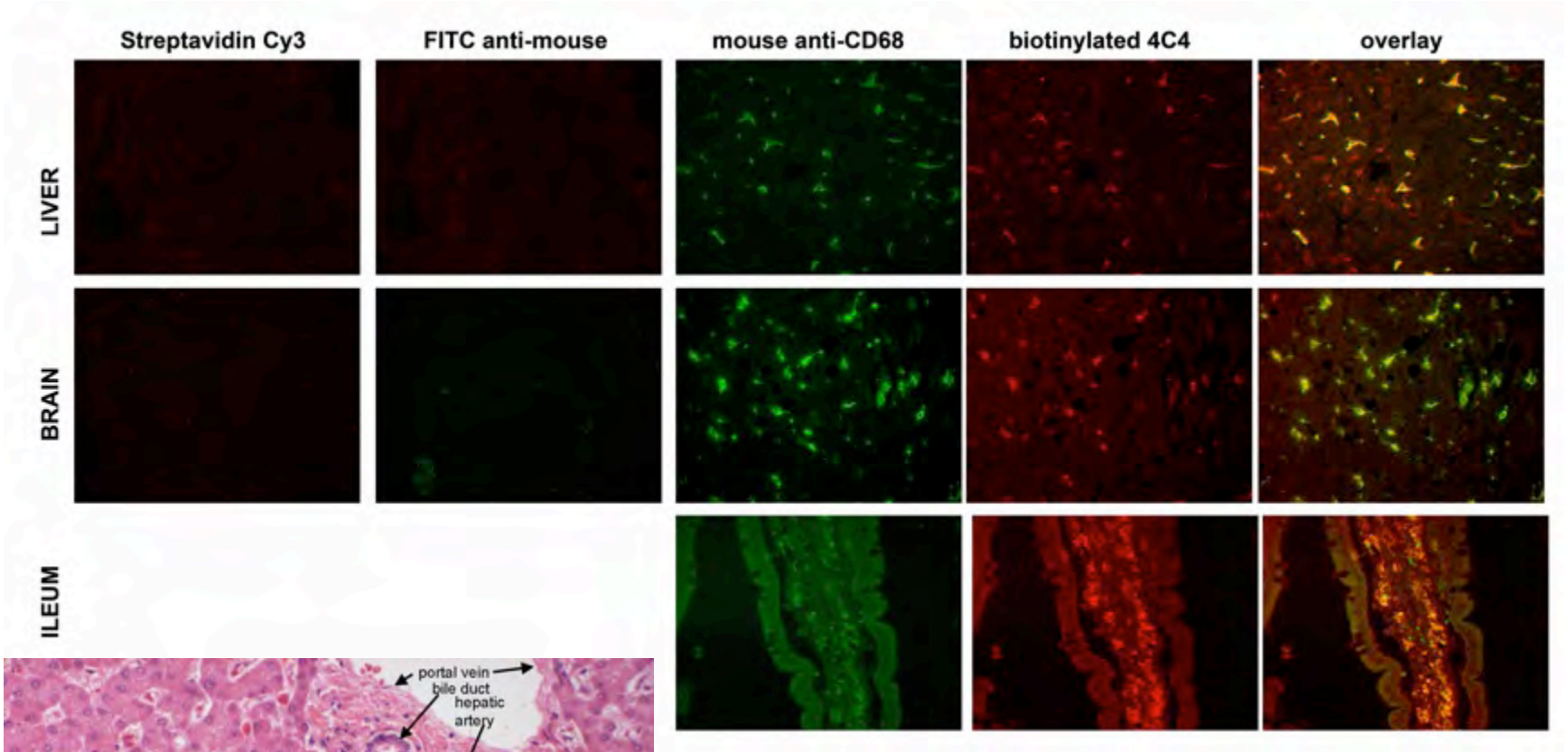
scale bar = 100 microns

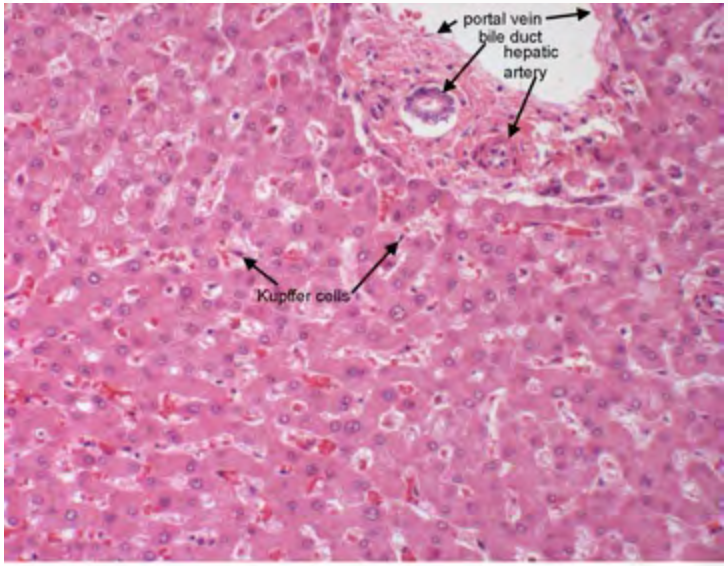
Microglia may be identified using:

anti-CD-68 (frozen or paraffin sections)

or **anti-Iba1 (paraffin sections) with spleen control**

Double label immunofluorescence to show co-localization with macrophage marker



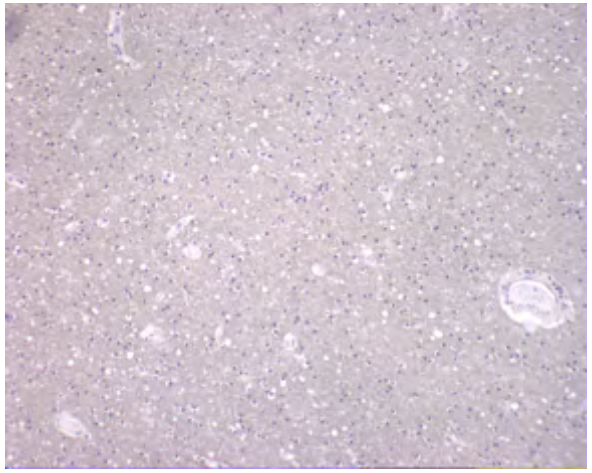


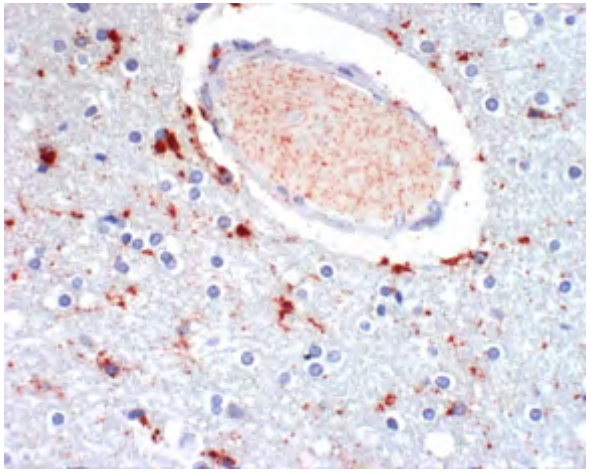
X63 (neg)

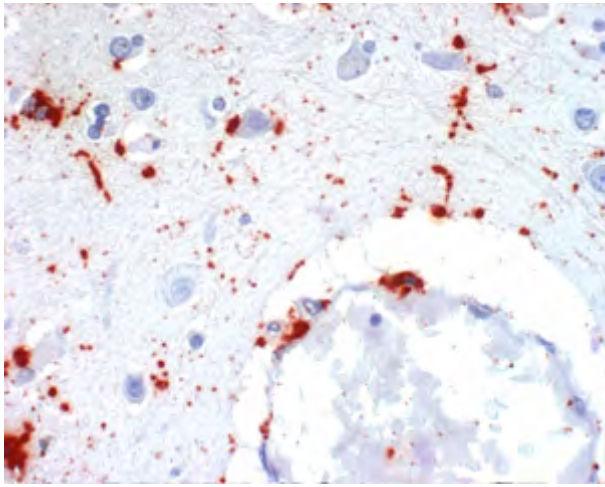
4C4 (Siglec11)

CD68(macrophages)

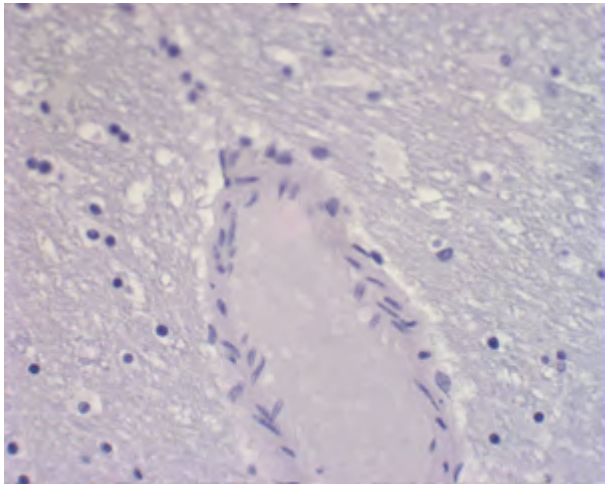
Human Brain

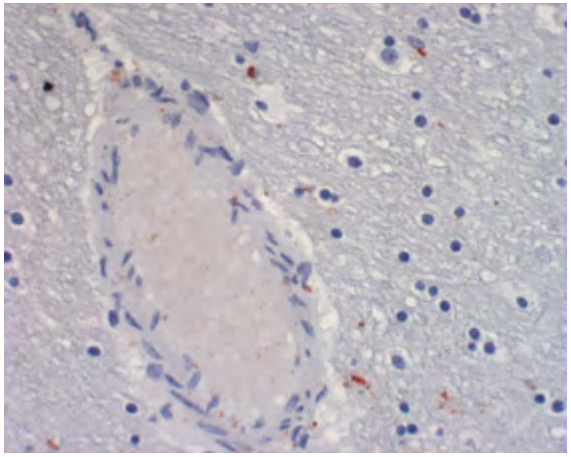


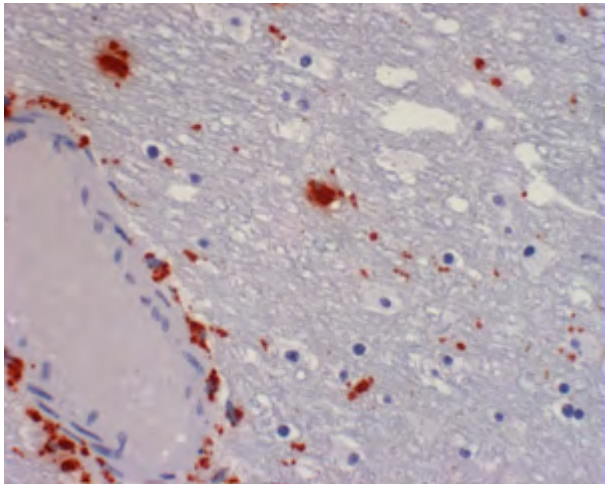




Chimp Brain

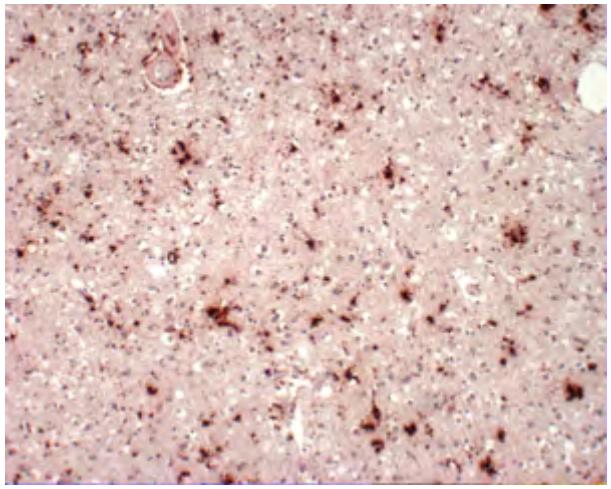


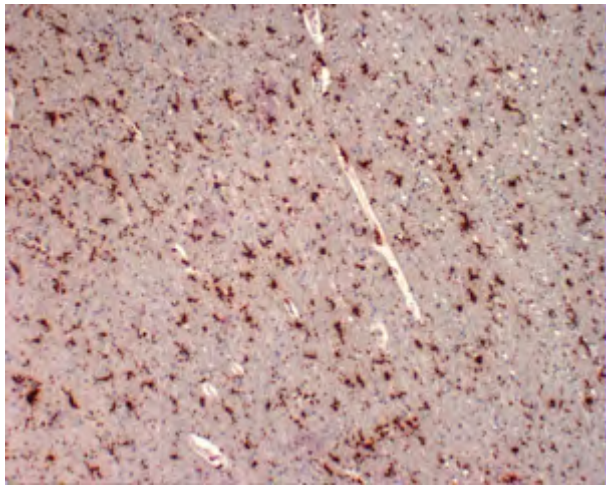




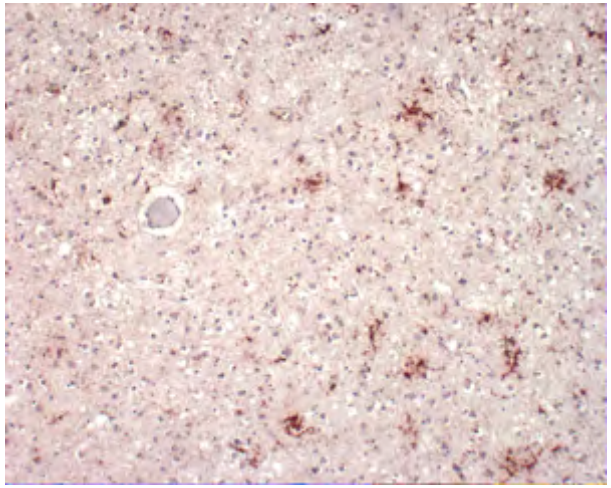
Hu Brain Alzheimer's HuBrainHIVencephalopathy

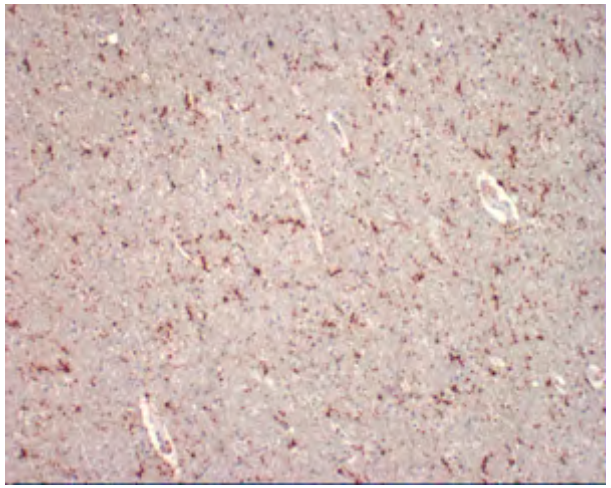
CD68





4C4



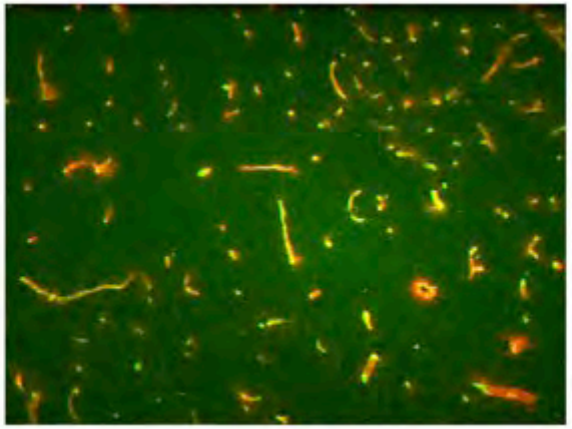


Increased levels of microglia and clustering

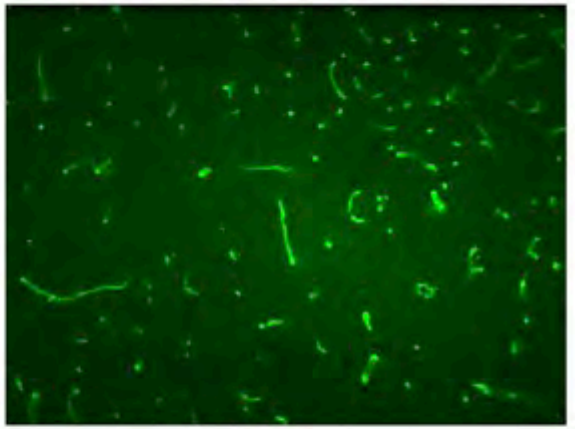
FITC anti-Chicken

co-localization to endothelial cells

overlay

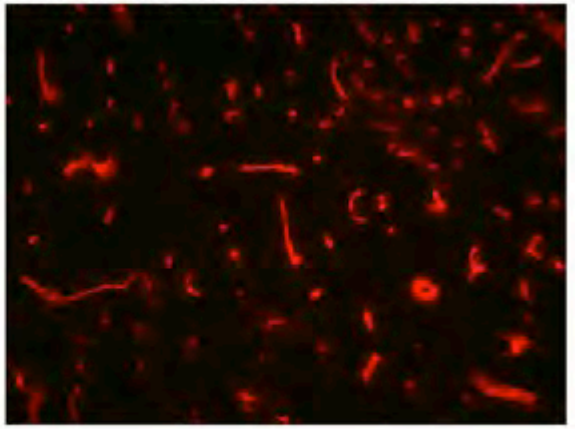


chicken anti-Neu5Gc



Cy3 anti-Rat

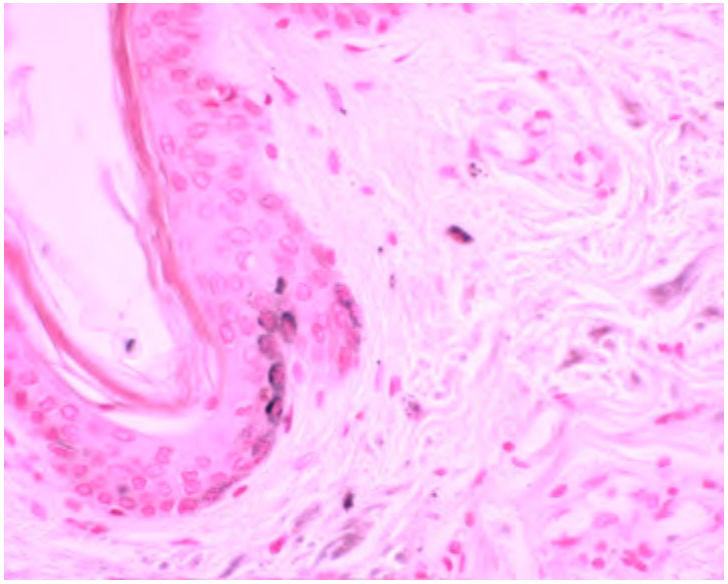
Rat anti-mouse CD31

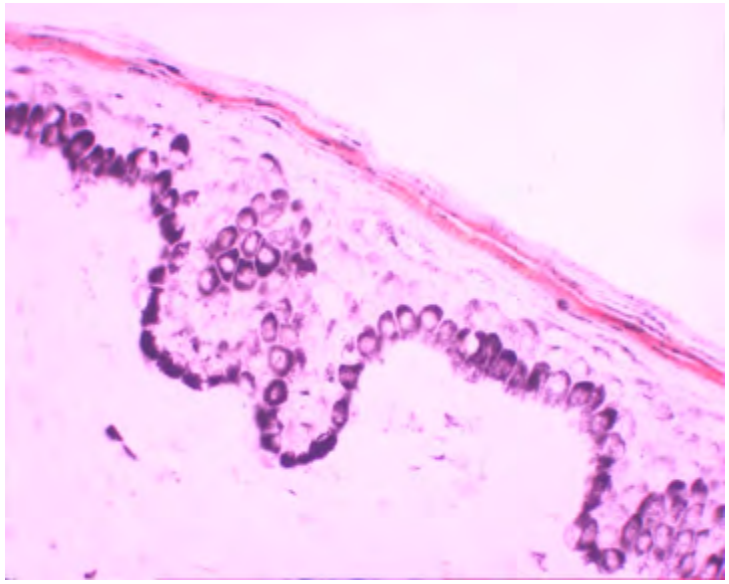


Co-localization and detection of similar epitopes on the same tissue section, using fluorescent markers

Neuroectoderm: after recruitment from the Ectoderm, this differentiates into the brain and spinal cord and cells from the neural crest migrate to:

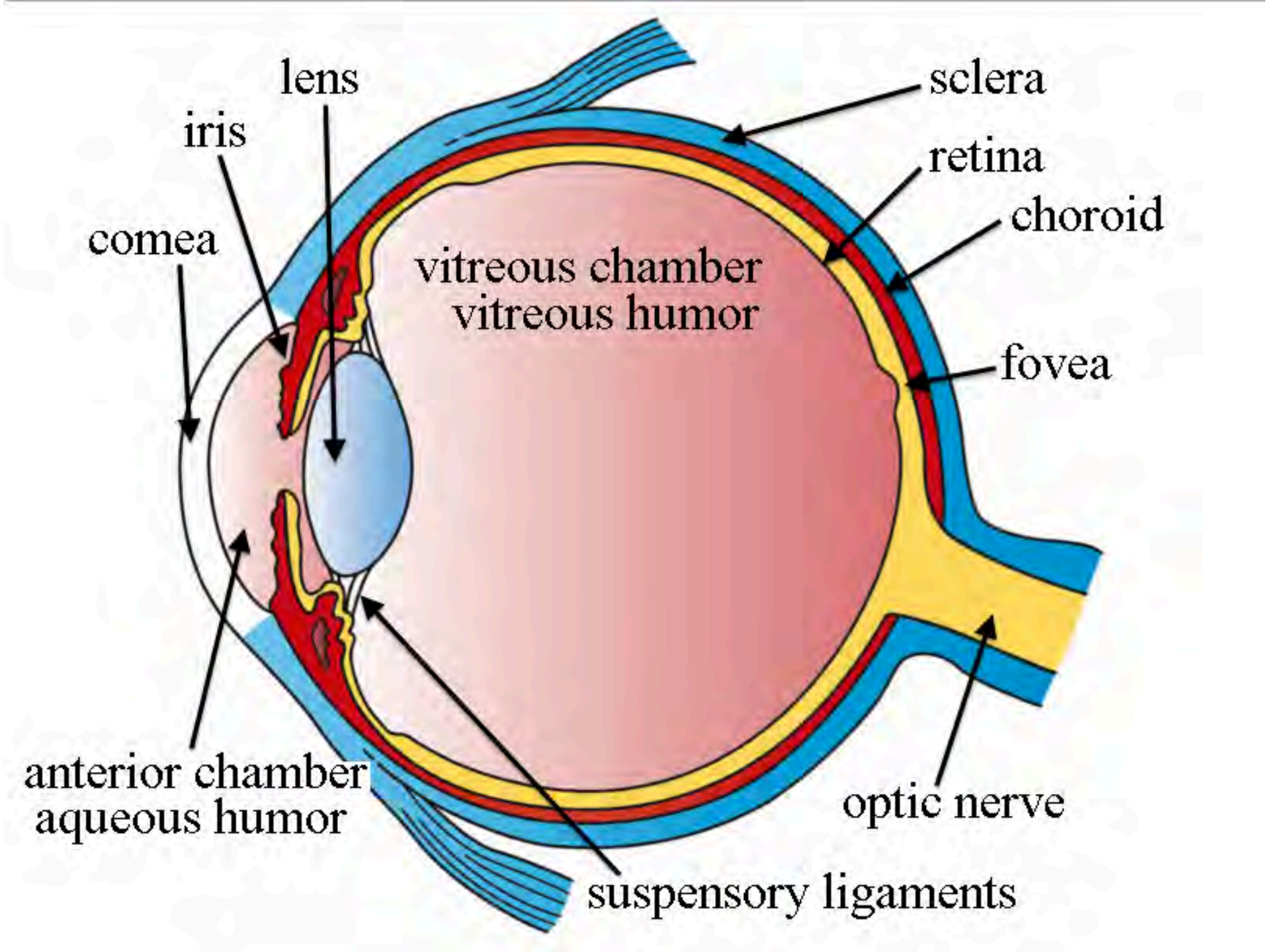
- **Skin—melanocytes**
- **Neuroendocrine cells**
- **Adrenal medulla**
- **Retina**

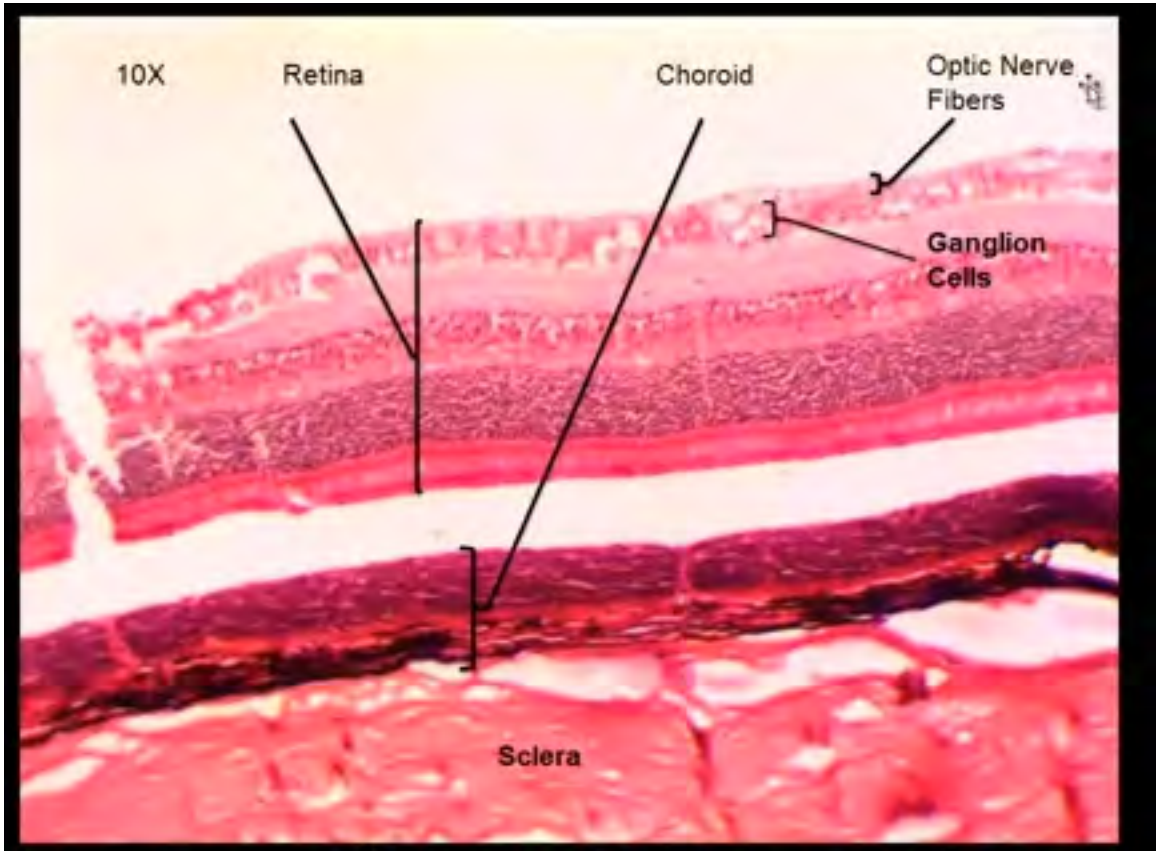


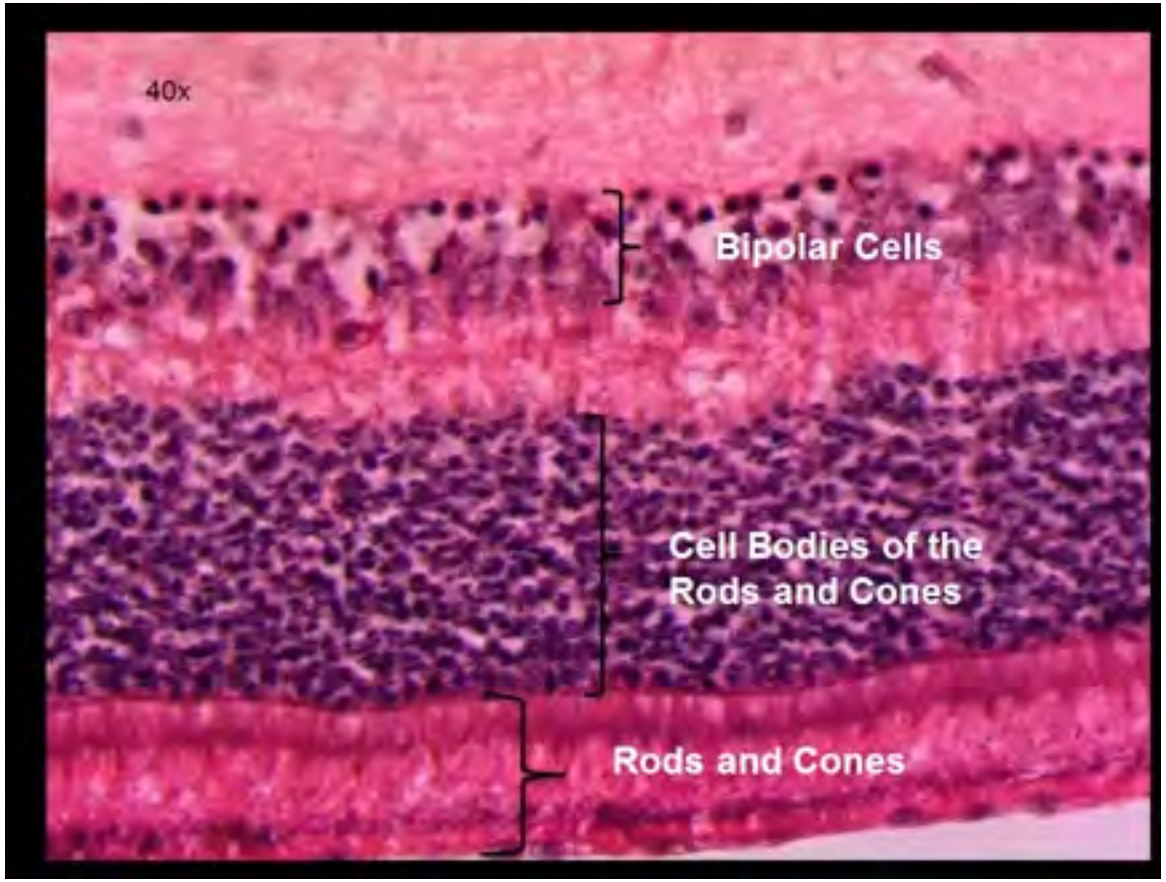


Melanocytes in basal layer of skin revealed with a silver stain

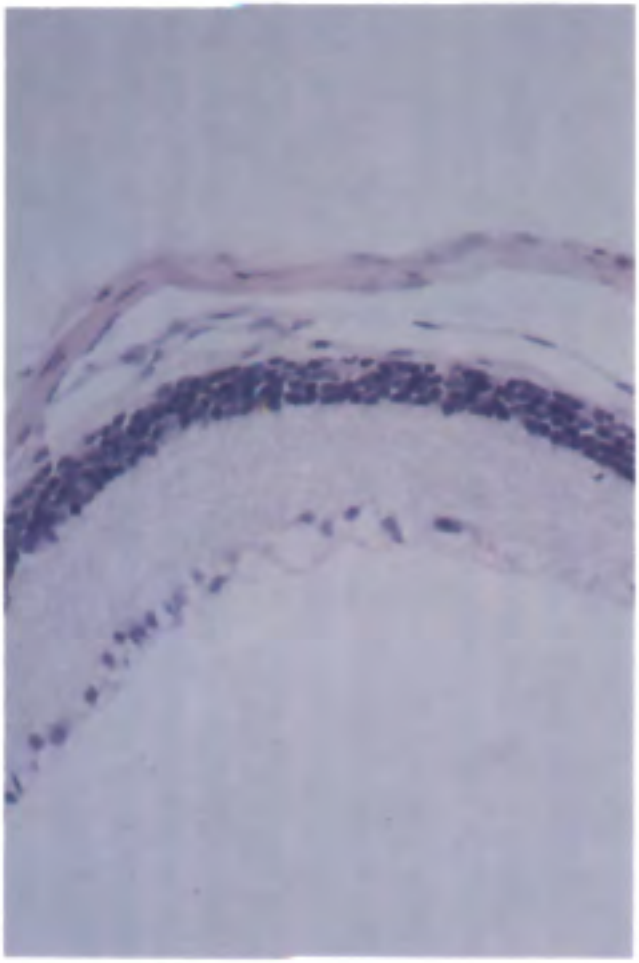
The difference in skin color between people of different pigmentation: number (quantity) of melanocytes is the same, but activity level is different (quantity and relative amounts of eumelanin and pheomelanin). This process is under hormonal control, including the MSH and ACTH peptides that are produced from the precursor proopiomelanocortin.
Tyrosinase, is required for melanocytes to produce melanin from the amino acid tyrosine.


lens
iris
comea
sclera
retina
choroid
vitreous chamber
vitreous humor
fovea
anterior chamber
aqueous humor
optic nerve
suspensory ligaments

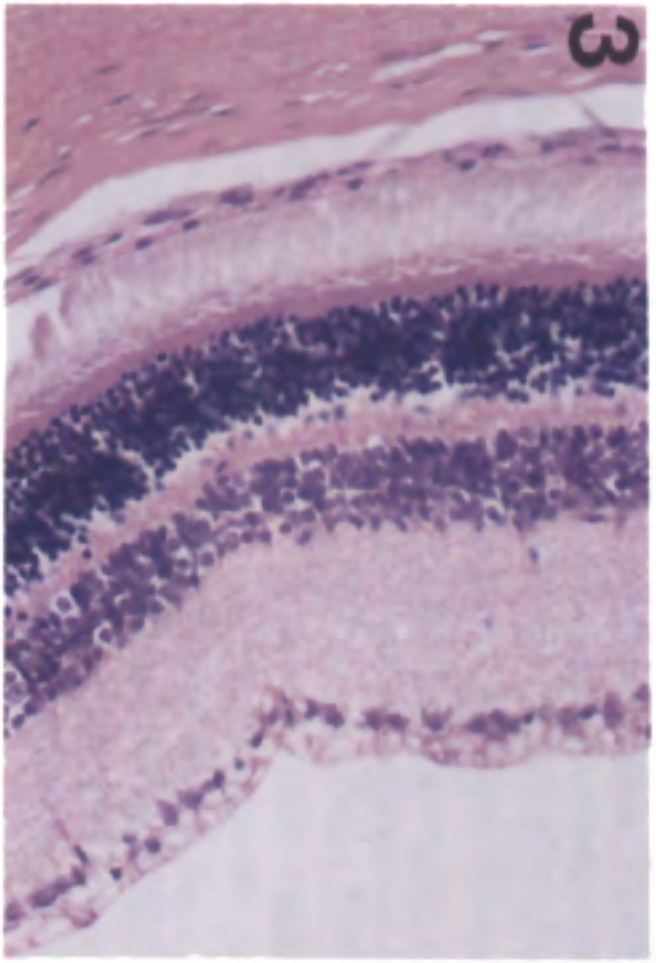

10X
Retina
Choroid
Optic Nerve Fibers
Ganglion Cells
Sclera

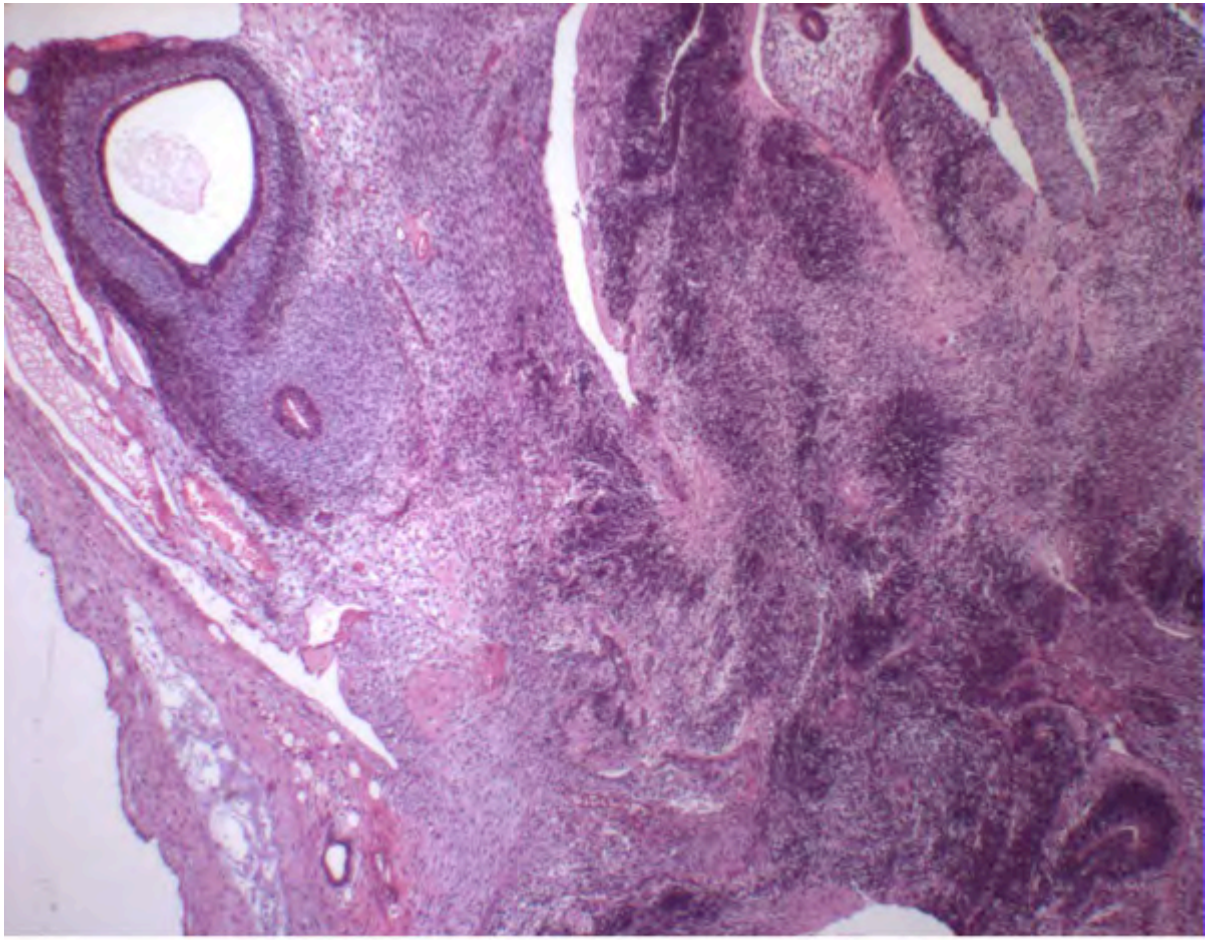

40x
Bipolar Cells
Cell Bodies of the
Rods and Cones
Rods and Cones

Abnormal retina in mutant



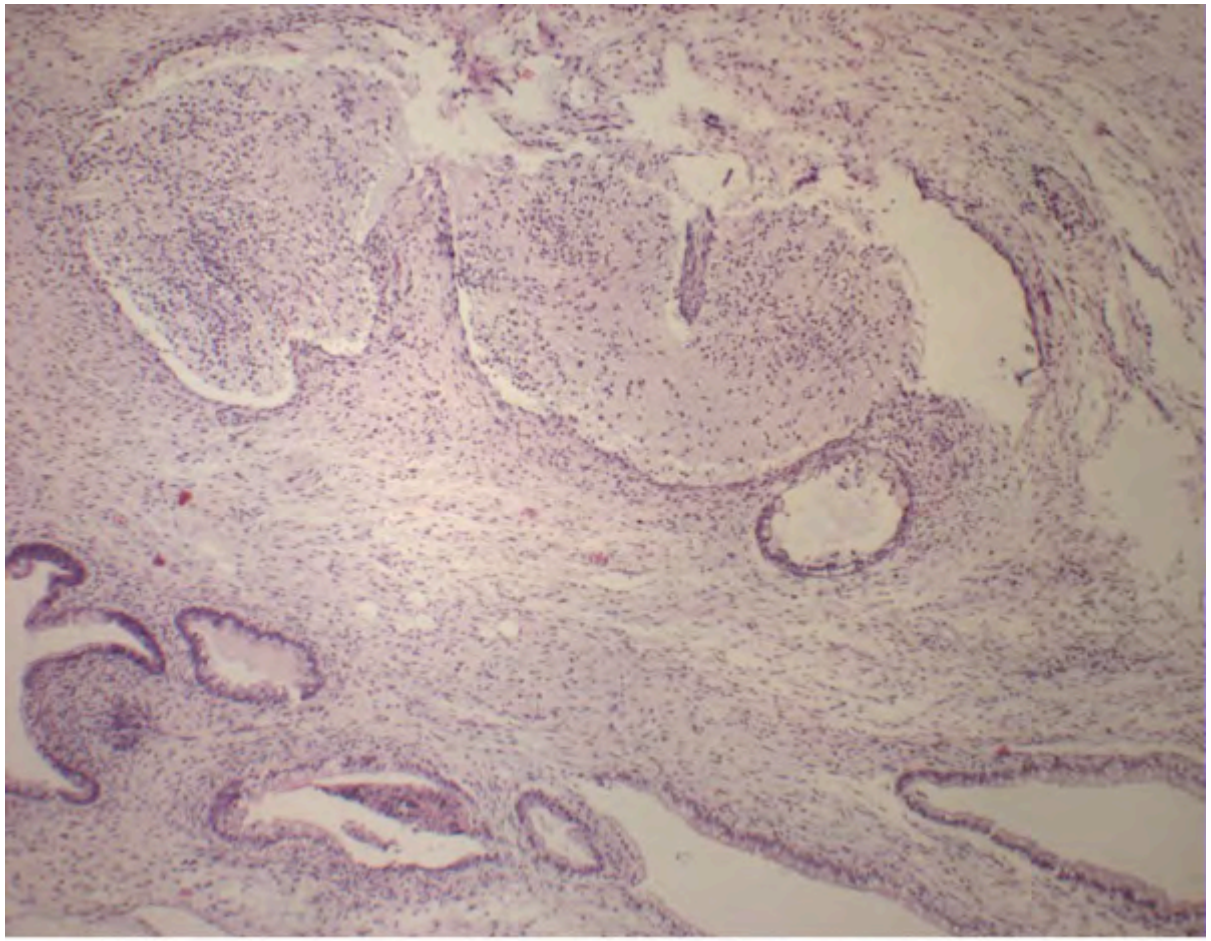
Control



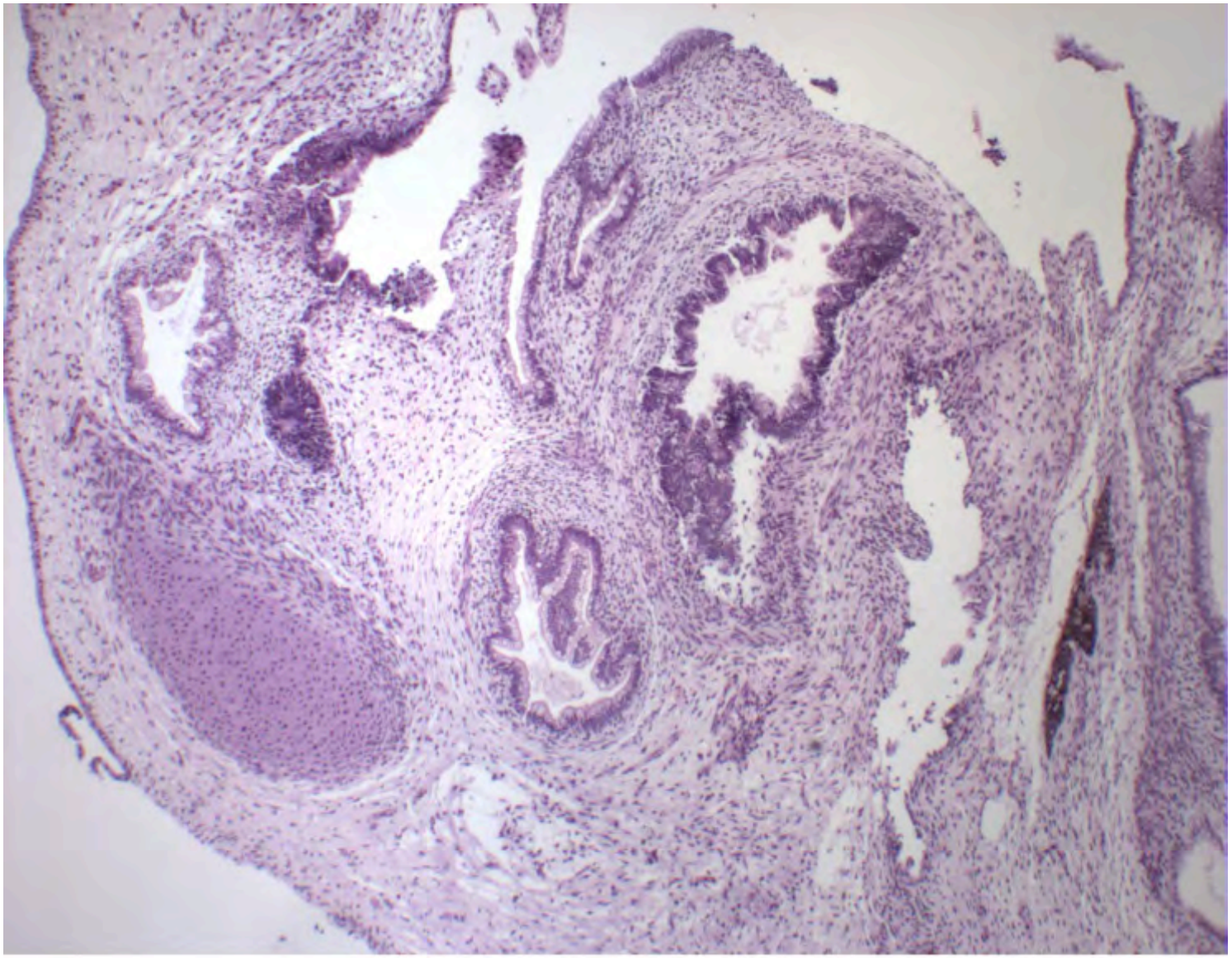


Implanted stem cells forming a teratoma—made up of cells from the 3 different compartments of embryonic tissue

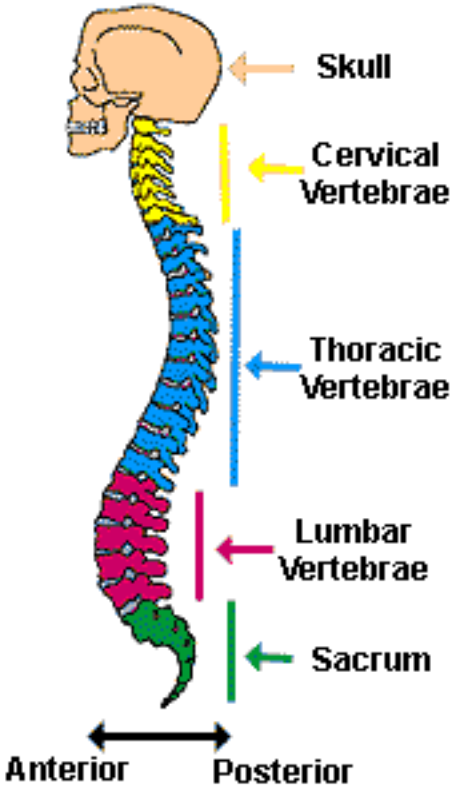
- Ectoderm (includes neuroectoderm)
- Endoderm
- Mesoderm

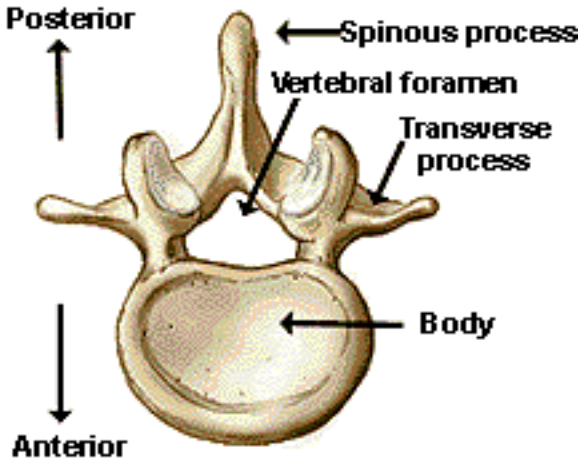


Teratoma

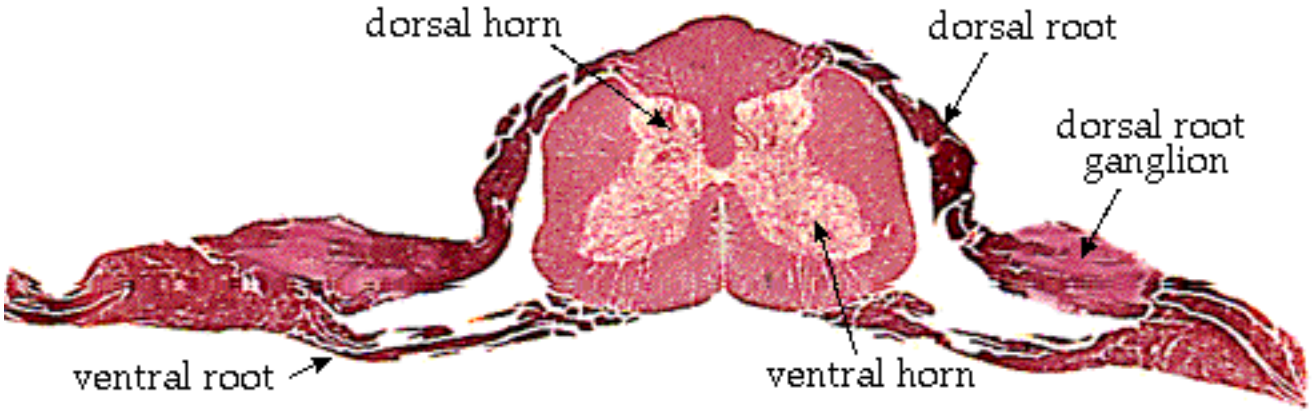


Teratoma



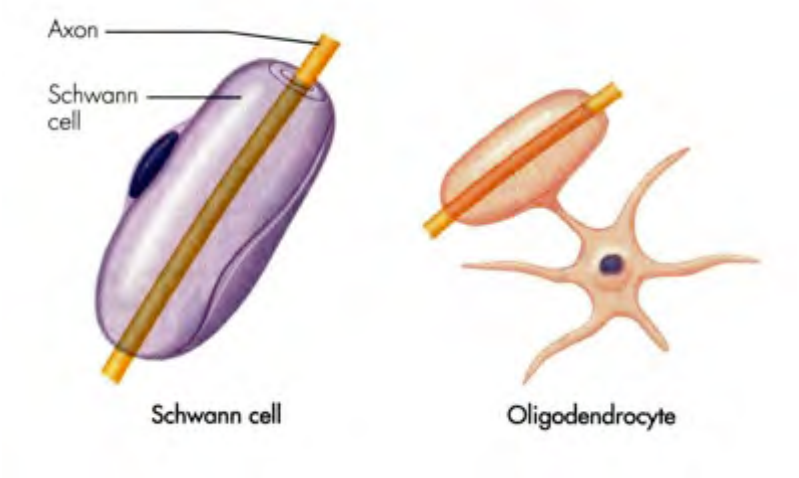


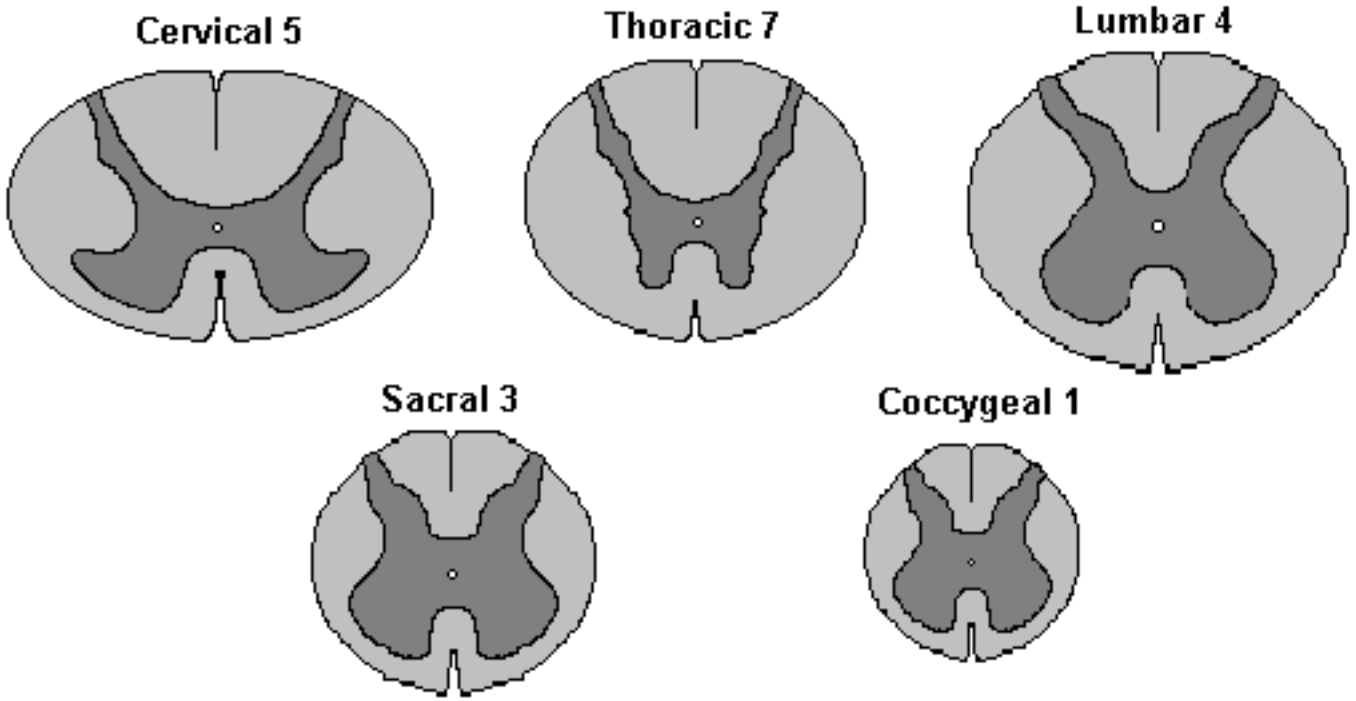
Although we usually study the spinal cord as a series of cross sections, it is important to remember that it is in fact a column, with continuous tracts and cell columns.



http://www.pathology.vcu.edu/WirSelfInst/glialpath.html

In the peripheral nervous system there are NO glia. There are Schwann cells which share one property in common with oligodendroglia, namely the production of myelin. Interestingly, the Schwann cells also become phagocytes, devouring the debris from injured peripheral nerves, and this property is not shared by the oligodendroglia

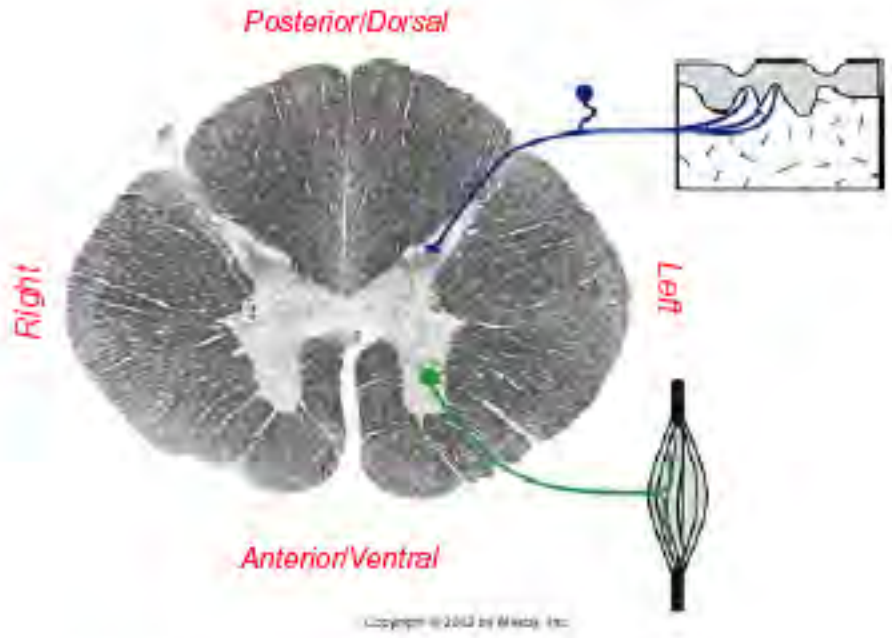




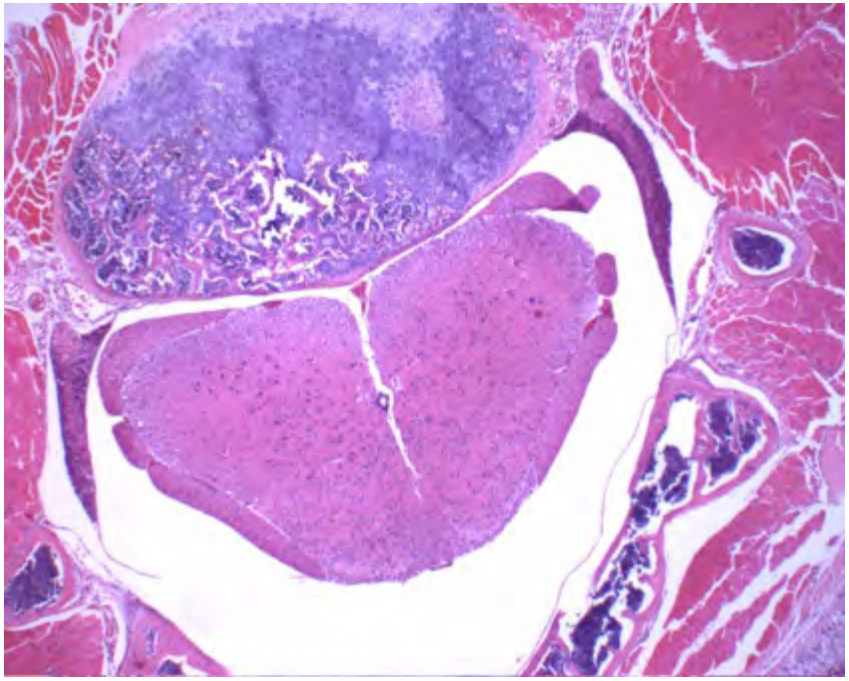
In the figures, note the differences in the shape and size of the spinal cord at different levels. The dark gray color in each segment represents "gray matter." If you use your imagination, you can see that the gray matter looks similar to an H or a butterfly. Nerve cell bodies are located in the gray matter. Surrounding the gray matter is white matter (lighter color shading) - this is where the axons of the spinal cord are located.

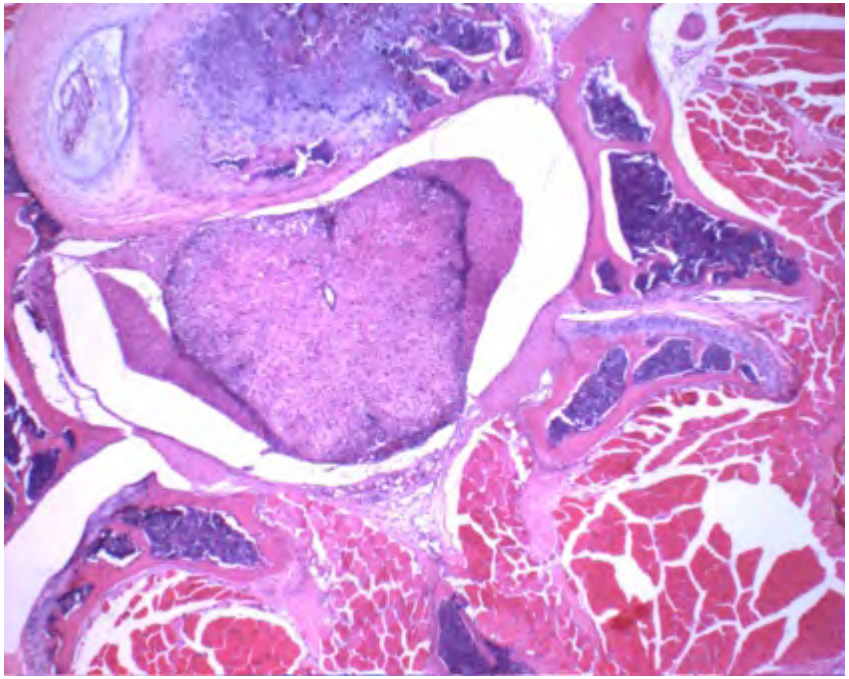
Only the ventral roots are coming out of the cord - the dorsal roots are actually going in. Throughout the cord, the dorsal grey matter (**dorsal horns**) deals with sensory perception, and receives information from the periphery through the dorsal root. The **ventral horns** contain the **α-motor neurons**, whose axons exit the cord via the ventral roots and travel directly to the muscles.

The Nervous System – Gray/White Matter in the Spine



Note: *Rostral* out of page, *Caudal* into page.





Spinal cord cross-sections to compare and determine if there are inflammatory infiltrates in the “treated” animal.

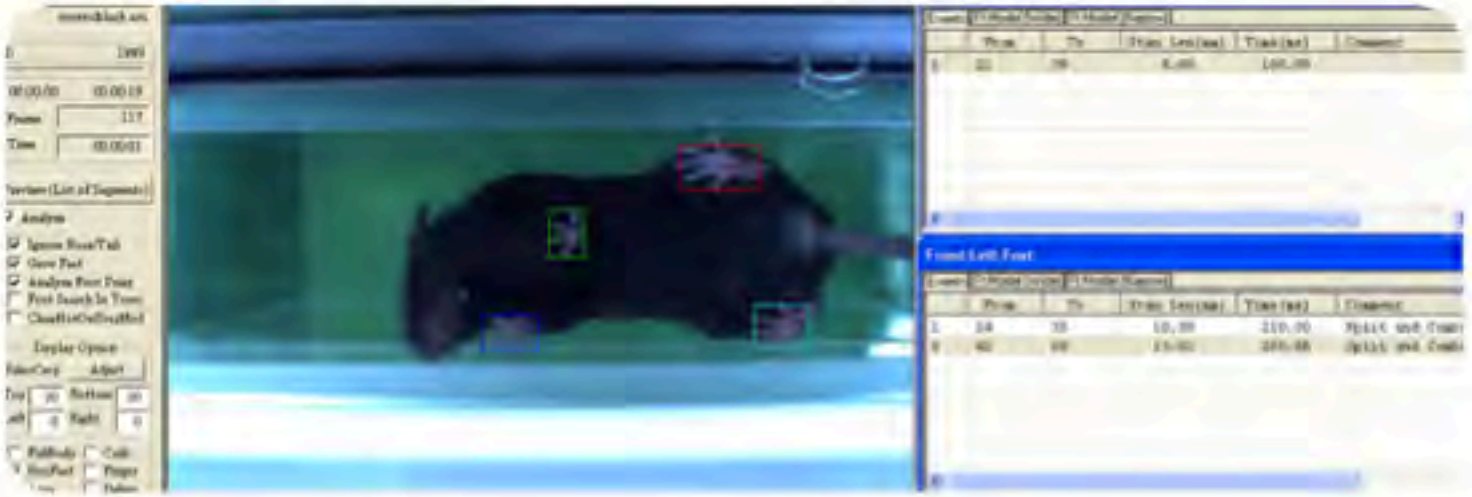
H&E x40

Metabolism changes and Behavioral analyses in mice

Metabolism and Behavior Core

The core performs a battery of tests on the live animals over a period of weeks. The tests include analysis and interpretation of: 3D Activity Levels, Food & water Consumption, Oxygen consumption, Carbon Dioxide Production, Circadian Rhythm changes, Measurements of Heart rate, Blood Pressure, Pulmonary Function, Fear Conditioning. Neurological Screen including Eyeblink, Tail Suspension, Rotorod tests, Balance Beam Stability and Wire Hang, Pole Test, Open Field, Tail Flick, Hot Plate Test, tests of Hearing, Prepulse Inhibition of Startle, tests for Social Dominance, Approaching Object, and Passive Avoidance.

Gait analysis

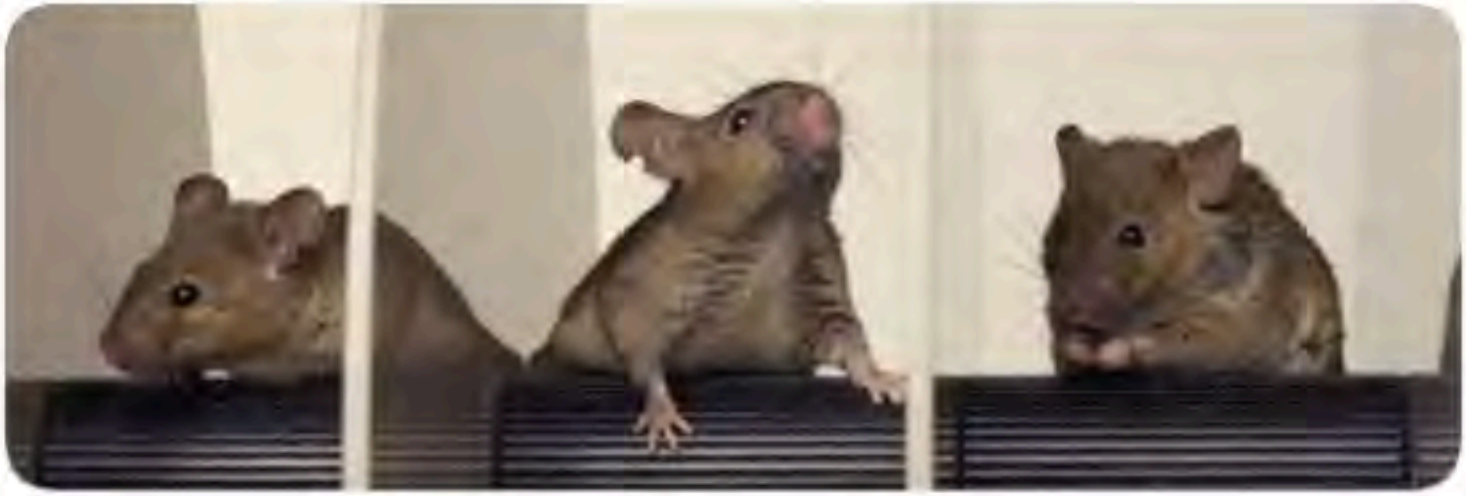


Gait abnormalities are seen in plenty of animal models. Gait disturbances can be assessed using a commercially available system comprising of a treadmill from Columbus instruments (Exergait, gait analysis treadmill) and the Treadscan software analysis from Clever Sys Inc. (USA). Mice are placed on a moving treadmill and gait is recorded via a high-speed camera placed underneath the moving belt. Each mouse is recorded and the moving motion is analysed to detect gait abnormalities. A number of correct steps need to be recorded per animal in order to get an accurate assessment. Several gait parameters are analysed, including stride and stance length and time or swing time.

Open Field

The OF test measures activity in a novel environment and can be used to assess a combination of locomotor activity, exploratory drive, neophobia, agoraphobia and other aspects of anxiety or fear in mice, as well as motor function. Testing sessions typically last up to 30 minutes. The apparatus consists of a perspex arena (approximately 44 cm _ 44 cm _ 50 cm high). The activity of the mice is assessed by the EthoVision video track system (Noldus Ltd.). Using the software, a centre zone (approximately 16% of the total area), a border area (an 8 cm wide border around the edge of the arena) and an intermediate zone (the remaining area) are defined. Quantitative parameters, such as the distance travelled and average speed, are recorded for the centre zone and the entire arena (see Eumorphia for the full Standard Operating Procedure). The test can be carried out under normal lighting conditions or under red light.

Rotarod



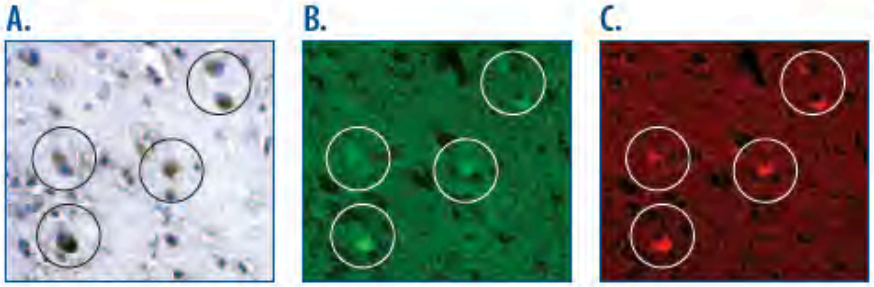
The Rotarod is used to assess motor co-ordination and balance. Up to 4 habituation sessions and 4 test sessions can be recorded per animal. The Rotarod apparatus consists of a rotating drum with a grooved surface for gripping. The speed of rotation can be set at a constant speed or can be set to accelerate at a particular rate. Under the acceleration mode, motor learning skills can be assessed. Mice are initially placed on the stationary drum for 1 minute and on the rotating drum (4 rpm lowest speed) for 3 sessions lasting one minute each with 10 minutes between each session. For test sessions, the rotating drum is set to accelerate from 4 to 40 rpm over 300 seconds. 30 minutes are left between training and test sessions and 15 minutes between each test session. After 4 sessions, the trial is ended and the mouse returned to its home cage. If, during a test session, a mouse slips from the drum within 300 seconds, it is returned to its home cage for 15 minutes before beginning the next test session.

Use labeled cassettes, to fix thin slices of organs or rolls of intestine, for at least 24 hours, before transferring to 70% alcohol, for processing into paraffin blocks

Do not leave brain samples in 70% alcohol for longer than 24 hours to avoid dehydration artefact in paraffin sections

Endogenous Tissue Background Control

Certain cells and tissues may have inherent biological properties resulting in background staining that could lead to a misinterpretation of the results. Before applying primary antibodies, cells and tissues should be inspected under the microscope using either fluorescence (for fluorescent labels) or bright-field (for chromogenic labels) illumination to ensure there is no signal inherent to the tissue itself. For instance, lipofuscin is an endogenous autofluorescent pigment that can be confused with positive staining.



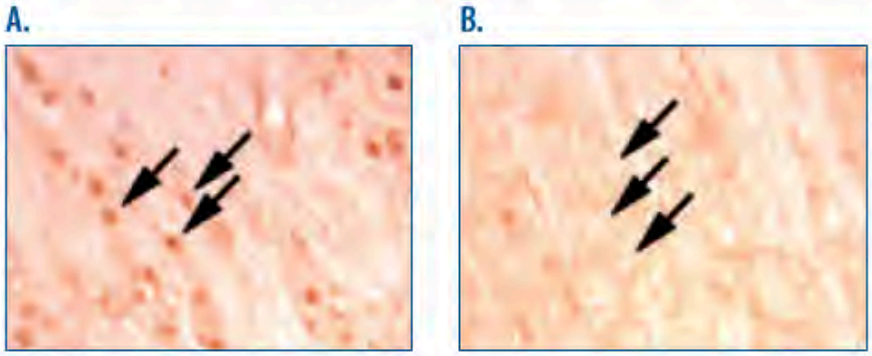
Lipofuscin Background in Nervous System Tissue. Lipofuscin is a pigment that accumulates with age in many tissue types. It also has autofluorescent properties that overlap with the excitation and emission spectra of commonly used fluorochromes. Circled in the micrographs above are lipofuscin-containing neurons that may appear labeled using either bright-field microscopy (**A**) or fluorescence microscopy in the green (**B**) and red spectrums (**C**).

No Primary Antibody Control

A control in which the tissue is incubated with antibody diluent, without the primary antibody included, is always necessary. This is followed by incubation with secondary antibodies and detection reagents. Staining with detection reagents alone should be negligible to the point that it does

The antigen to antibody mixture should be made at a working dilution of 10:1 (molar ratio) and be pre-incubated overnight at 4˚C. The pre-absorbed antibody can then be incubated with tissue in place of the primary antibody alone. The staining pattern produced by the primary antibody can be compared to that produced by the pre-absorbed antibody.

Absorption controls work better if the immunogens are peptides. However, if antibodies were raised against the whole protein, addition of the mixture of antibody plus protein may result in higher non-specific staining. Although the mechanism is unclear, the antigen used for pre-absorption may itself bind to the tissue and result in non-specific staining. Thus, it is important to note that an absorption control using whole protein may not always confirm the specificity of an antibody for the protein in the tissue.



Absorption Control in Rat Dorsal Root Ganglion. A. A cryostat section of rat dorsal root ganglion stained for phospho-MSK1 (S212) using anti-human Phospho-MSK1 (S212) affinity purified polyclonal antibody (Catalog # AF1036). **B.** Nuclear staining (indicated by arrows) is abolished if the antibody is first pre-absorbed with the S212 phosphorylated immunogen.

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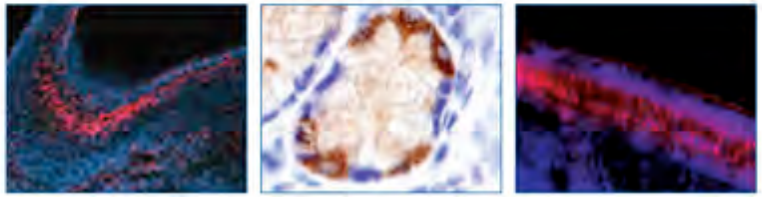

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Immunohistochemical Staining Methods

Design controls for secondary and/or tertiary reagents

A. Reagent controls should include:
   1. Slide that receives diluting buffer alone
   2. IgG control at the same concentration as the test antibody
   3. Positive control reagent, same species as primary being tested

B. Tissue / or cells controls should include:
   1. Tissue or cells not expected to be positive
   2. Tissue or cells expected to be positive
   3. Blocking reagent to delete positive reaction,
      to demonstrate specific binding by the test antibody

When testing a new antibody, one needs to know:

Species of origin of the primary antibody: mouse, rabbit, rat, hamster, chicken, goat, sheep, horse……..

In order to :

---- design what cells or tissues will be used as positive and negative controls

---design secondary and tertiary detecting reagents

----design reagents to block non-specific binding

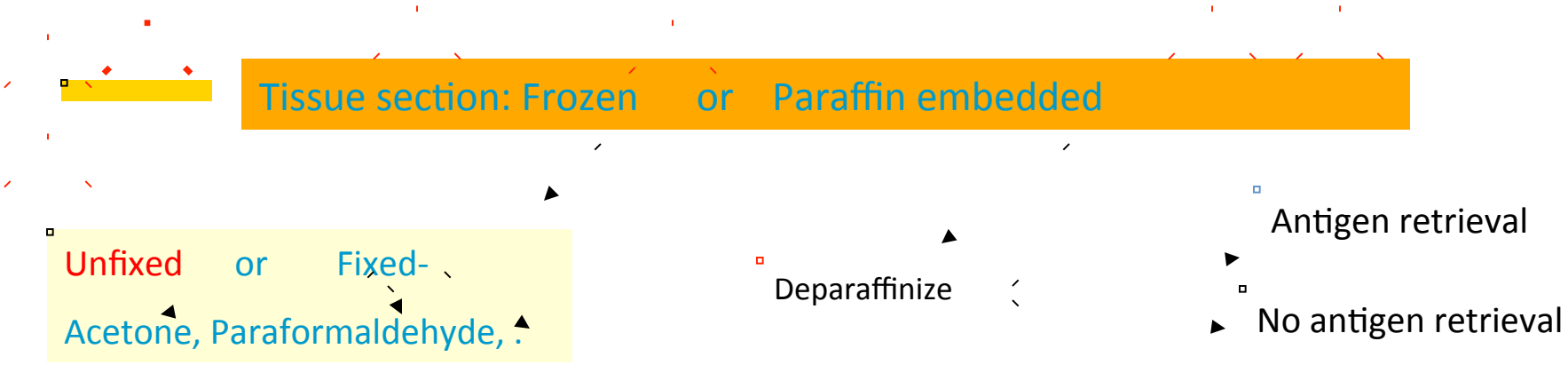
Primary antibodies may be

Polyclonal

(rabbit, sheep, goat,chicken)

Or Monoclonal

(rat, mouse)



At each step of the immuno assay , if using new reagents,

one needs to determine the optimum working dilution

Unbound antibody washed off before application of secondary reagent

Primary antibody : maybe used, already labeled with fluoresceinated compounds or with an enzyme label

Block nonspecific binding sites before adding primary antibody / reagent

Tissue section: Frozen or deParaffinized

Block Non-Specific Binding sites **in tissues, because of the large variety of cells present**

--**Block non-specific binding to extra cellular matrix components,** usually use bovine serum albumin

if using HRP conjugates Block endogenous peroxidases **in RBCs present in all tissues**

If using alkaline phosphatase conjugates , endogenous alkaline phosphatase in tissues will contribute to annoying background binding eg: within sections of frozen intestine , Bone marrow, placenta. This may be removed using heat or O.1M glycine

if using biotinylated secondary reagents Block endogenous biotin
There is endogenous biotin in most tissues other than spleen or thymus

--Treat one set with block to prevent binding of first reagent

Unbound reagent is washed off before application of next reagent

Secondary reagent,:

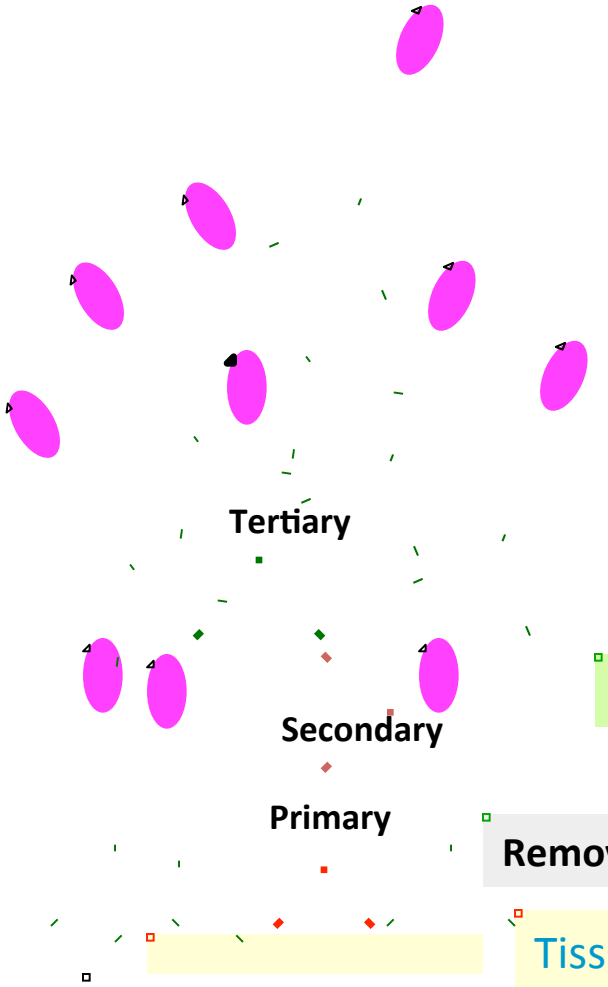
may be used already labelled with

fluoresceinated compounds or with an enzyme label

OR **it may be conjugated with Biotin, digoxygenin, etc.**

Dilute secondary reagent with normal serum of species being tested to block nonspecific binding of secondary

Tissue section: Frozen or deParaffinized



DAB, AEC, red , SG, VIP

Blue, Red (also fluoresces)

HRP Alk.Phos **or with an enzyme**

AMCA

CY2 , FITC

PE, CY3

fluoresceinated compounds

Tertiary reagent is used usually labeled with :

Remove endogenous binding sites in tissue to prevent nonspecific binding

Tissue section: Frozen or deParaffinized

Wash off unbound tertiary before adding substrate or before mounting to view or before further amplification

Using double stain methods to determine if two antibodies recognize the same epitope on cells in a tissue section

Need to use lower dilutions of antibodies when doing a double stain to compensate for additive effect

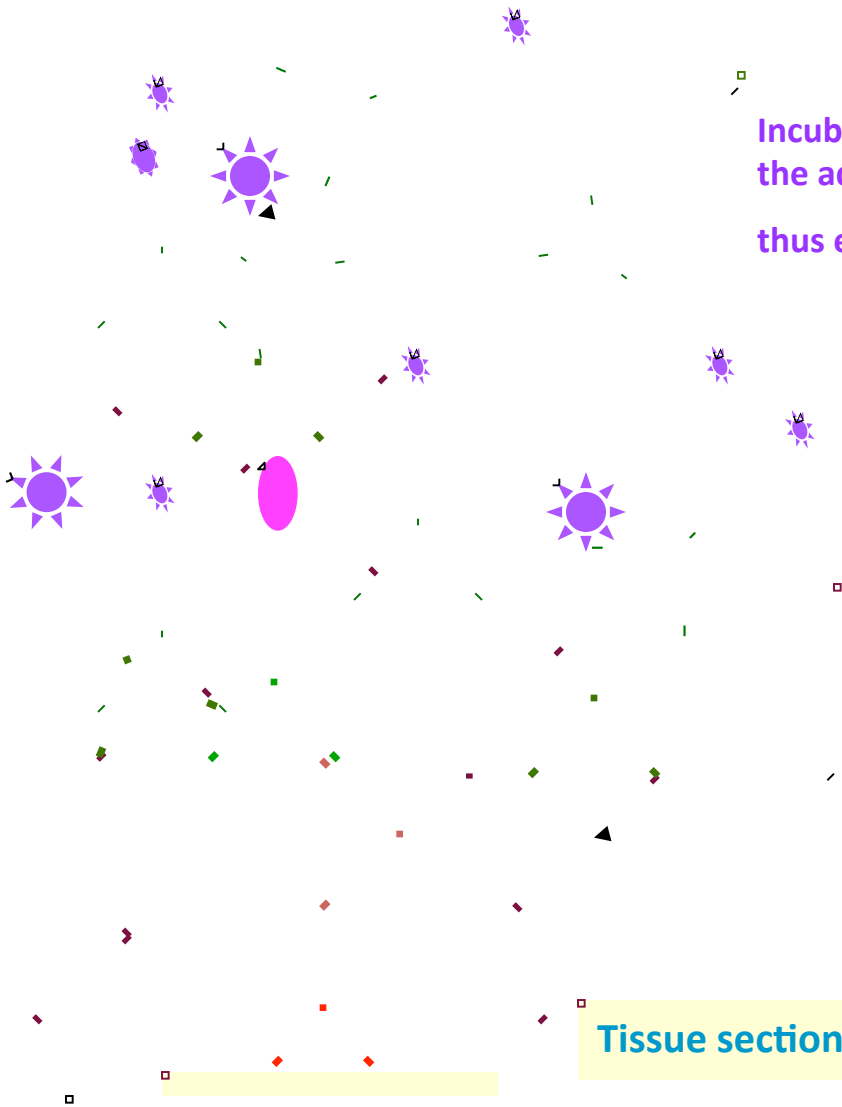
May use antibodies sequentially and detect each with different fluoresceinated tags so that if the same epitope is recognized a new color will be visualized

The second antibody may bind to the same epitope within cells that are less abundant

One antibody may bind with higher affinity or recognize an epitope within cells that are more abundant

Tissue section: Frozen or deParaffinized

An example of further Amplification to detect low abundance epitopes in tissue



Incubation with more labeled streptavidin then detects the additionally generated biotin,

thus enhancing detection levels by a factor of 100-1000

Biotinyl tyramide uses the HRP enzyme

to deposit many biotin molecules that "cover"

the antigen antibody complexes

already formed over the epitope on the tissue section

Tissue section: Frozen or deParaffinized

Wash off unbound before developing or mounting