

## PCB 4023 – Cell Biology

### Lab 6: Nervous Tissue

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**N.B.** Since this document is in “pdf” format, the URLs (web addresses) cannot be linked. To use them, simply highlight and copy the address and paste it into the address box of your browser.

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#### **Preparation assignment** (to be completed before lab (1/25/02):

- 1) Kerr (1999) Chapter 5. Muscle (pp. 81-106).

#### Web resources:

**N.B.** Again, you may wish to examine the modules pertaining to muscle at the University of Florida College of Medicine histology tutorial. There are separate review modules and on-line quizzes for nervous tissue and special sensors. For nervous tissue there is a review module and an on-line quiz; a separate module and quiz is available for special sensors but we will touch only lightly upon this. As before, these modules may contain information that is not covered in this course and you will not be held responsible for any information presented there. However, you may wish to consider using them as additional preparation and/or review. The URL is as follows:

<http://www.medinfo.ufl.edu/year1/histo/index.html>

Know of any other web sites pertaining to the nervous system that you have found helpful or interesting? E-mail me the link at [condon@fiu.edu](mailto:condon@fiu.edu). and let me know what and why you found it informative and/or interesting.

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## The Nervous System

Recall that there are 4 major basic types:

- 1) epithelia - continuous layers of cells with little intercellular space which line surfaces and form glands
- 2) connective tissue - cells embedded in abundant extracellular matrix
- 3) muscles - specialized contractile cells with electrically excitable membranes
- 4) nervous tissue - consists of (1) electrically excitable neurons and (2) their support cells (neuroglia or glia)

The nervous system is the organ system concerned with processing information from the environment and using that information to control the physiology and behavior of the organism. The nervous system has three basic components: (1) Sensory input by sensors which transduce stimuli into electrical signals; (2) integration components which decide what to do with this information; and (3) motor components which direct the response to effector organs (e.g., muscles, glands, etc.).

Anatomically (but not functionally), the nervous system can be divided into two subdivisions: (1) the central nervous system (CNS) consisting of the brain and spinal cord and

derived from the neural tube; and (2) the peripheral nervous system (PNS) consisting of (a) all the nerves that emanate from the CNS (spinal and cranial) and (b) the peripheral ganglia and the nerves originating from them. In contrast to the CNS, neurons within the PNS are derived from the neural crest and neural placodes. In the CNS collections of axons bound together with connective tissue are called tracts whereas these are termed nerves in the PNS (thus, paradoxically, there are no nerves in the central nervous system). Similarly, a group of neuron cell bodies in the CNS is called a nucleus whereas in the PNS such a structure is called a ganglion.

Macroscopically, the CNS can be observed to consist of gray and white matter. Gray matter is dominated by neuron cell bodies and white matter consists chiefly of tracts (the white appearance is due to the lipids of the myelinated axons). In the spinal cord, the gray matter [which in fact is lighter than white matter] is internal to the white matter and in transverse sections takes the shape of a butterfly with dorsal and ventral horns. The dorsal horns contain neurons receiving information from sensory neurons whose cell bodies are found in ganglia (e.g., spinal) and whose axons form the dorsal root to enter the spinal cord. Conversely, the ventral horns contain the motoneurons whose axons from the ventral roots and convey information to the effector organs in the periphery (glands and muscles). Also with the gray matter are interneurons which, as their name suggests, are neurons which connect to other neurons. Peripherally, the ventral and dorsal roots converge to form spinal nerves.

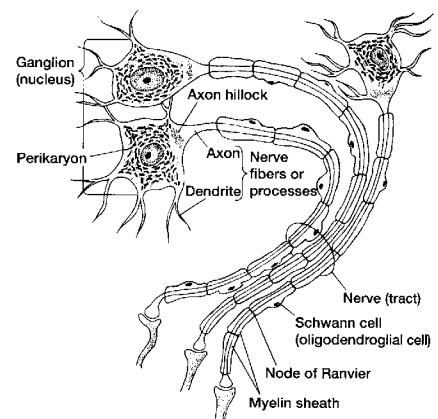
The macroscopic structure of the brain is a good deal more variable than in the spinal cord and cannot be so simply summarized. In general, the gray and white matter is more intermixed although in the cortex of the cerebrum and cerebellum the gray matter is external and the white matter internal. [The good news is that the gray matter in the brain is grayer and the white matter is whiter.]

Surrounding, supporting and protecting the CNS are three concentric connective tissue layers called the meninges (Gr, membrane). From superficial to deep these are (1) the dura mater (L, tough mother), (2) the arachnoid mater (G., spider-like mother), and (3) the pia mater (L, tender mother). As their names suggest, the dura is the thickest and toughest layer, the arachnoid mater is wispy, resembling the silk threads of a spider's web) and the pia mater is adherent to the underlying nervous tissue (like a mother to her infant; Gag me with a spoon!). Cerebrospinal fluid (CSF) and circulatory vessels run in the space (subarachnoid) between the arachnoid and pia maters.

figure: 3K16-2.tif , 3K16-3.tif

Nervous tissue is comprised to two classes of cells, both of ectodermal origin: (1) neurons, cells with excitable membranes and (2) neuroglia or glia (L., glue), support cells . Neurons forms the functional units of the nervous system and are electrically excitable cells whose membranes can undergo changes in charge. Reversal of this charge across the membrane, the nerve impulse or action potential, is the language by which neurons communicate.

The majority of neurons possess 4 anatomically distinct regions: (1) Dendrites (Gr, tree) are the processes which receive signals from other cells; (2) the soma (or perikaryon or body) integrates information coming from the dendrites, generates the action potential (in most neurons) and is also the biosynthetic center; (3) the axon is the process that conducts impulses away from the soma, and are of variable length (motoneuron axons can reach meters in length); and (4) the axon terminal. Axon terminals are usually multiple, the axon typically dividing into multiple branches next to its target cell(s). The tips of the axon terminals contains the synapses, electrical or chemical junctions between the



neuron and its target cell.

Different types of neurons differ in their elaborations of these different regions and reflect their diverse functions. For example, neurons important in receiving and integrating information (e.g., Purkinje cells of the cerebellum) typically have elaborate dendrites.

figure: 3K16-2.tif , 3K16-3.tif

Neuroglia or glia support, nourish, and insulate the neurons. Unlike neurons, their cell membranes are not electrically excitable. In the central nervous system (CNS) glia cells typically outnumber neurons 10:1. In addition to their support role in mature tissue, glia play an important role in development of the nervous system by acting to guide axons to their proper targets.

In the CNS there are four types of glial cells: (1) Astrocytes pass nutrients between capillaries and neurons and form the infamous blood-brain barrier; (2) microglia are phagocytic cells which engulf foreign material and bacteria; (3) oligodendrocytes provide the myelin sheathing for the tracts of axons; and (4) ependymal cells are epithelial like neurons lining the central canal of the spinal cord and brain (ventricles).

In the peripheral nervous system (PNS) there are two types of glial cells: (1) Satellite cells provide nutritional support and (2) Schwann cells provide myelination for the axons of nerves.

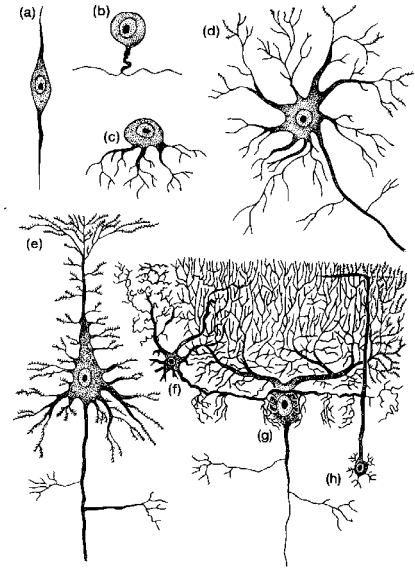
### Lab Assignment: Muscle

Work through the following sections using your atlas as a guide. Make sure to answer the questions (marked by “?”) at the end of the lab; these will be evaluated when you turn in your handout next week. A list of structures which will form the basis for next week’s quiz is given at the end of the handout.

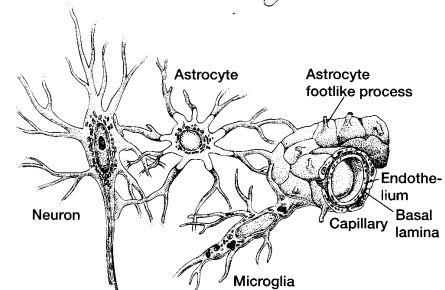
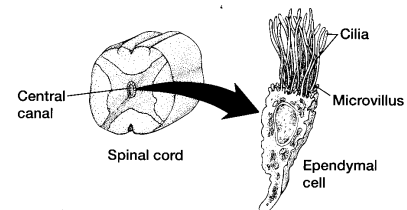
To learn how to identify the structures, write down criteria which will assist you in your identification (e.g., simple squamous epithelium: single layer, flat cells with flattened nuclei). Your text is a good source for such material as well as your own observations. Some students find it helpful to make rough sketches of the structure to assist in their learning.

**N.B.** Due to [unprogrammed] slide death, your slide box may not contain the required slide. If this is the case, notify an instructor and they will provide a replacement or suggest an alternative. If you end up borrowing a slide from one of your colleagues’, please don’t forget to return it to them.

Many aspects of histological identification in nervous tissue requires specific staining techniques, most of which are not represented in your slide set. For example, differentiation of the different types of neurons and glia within the CNS requires that the entire cell be demonstrated, particularly the processes. This can only be done with silver stains (e.g., Golgi) but the technique is notoriously fickle (only a subset of the cells pick up the stain; problems with



**FIGURE 16.3** Types of neurons. (a) Bipolar neuron. (b) Unipolar neuron. (c-h) Multipolar neurons.



overdevelopment) and good slides can be difficult to obtain (e.g., your slide set). Nissl staining (Nissl substance is simply condensed endoplasmic reitculum produced by formalin fixation) using either cresyl violet or thionin is useful in distinguishing neurons from glia, but is of limited use in distinguish types of neurons as only the soma is labeled. Myelin is best demonstrated in frozen sections (rather than paraffin in which lipids are removed during dehydration) using osmic acid (osmium tetroxide) which reduces lipids to a black compound. Weigert's myelin sheath stain use ferrous hematoxylin to stain myelin blue and is routinely to study gross sections of the CNS (tracts are stained dark; nuclei appear white).

## I. Spinal cord

spinal cord: H1550 (silver; extras available), HE 2-21 (faded), HE 2-24 (best)

In the gray matter note that the dorsal horn extends closer to the surface than does the ventral horn. In the ventral horn the largest cells will be motoneurons whose nuclei are pale and contain distinct nucleoli. Ependymal cells line the central canal. The cells in the white matter are predominately glial. Note also the axons and myelin anuli around them. The groups of axons appearing to emanate from the dorsal horn are the dorsal roots and those from the ventral horn are the ventral roots. If you have led an exceptionally virtuous life, your slide may contain a spinal (dorsal root) ganglion whose pseudounipolar neurons give rise to the dorsal root and are supported by the glial satellite cells. Peripherally, the ventral and dorsal roots merge to form the spinal nerves.

Surrounding the spinal cord are the three connective tissue coats known as the meninges. The dura mater is the thickest and most superficial. The pia mater is the deepest and is adherent to the spinal cord while the arachnoid mater is the wispy like connective tissue in between the pia and dura maters.

Identify:

- gray matter
  - dorsal horn
  - ventral horn
    - motoneurons: soma, nucleus, nucleoli
  - ependymal cells
- white matter
  - axons
  - myelin anulus (pl. anuli)
  - glial cells
- roots:
  - ventral
  - dorsal
- spinal (dorsal root) ganglion (if you've lived a virtuous life)
  - pseudounipolar
  - satellite cells
- spinal nerve (if present)
- meninges
  - pia mater
  - arachnoid mater
  - dura mater

## II. Cerebellum

cerebellum, Golgi (silver) stain: 93W3623  
cerebellum, H&E: DEMONSTRATION

We'll continue our examination of the CNS by examining the cerebellum (L, little brain). Part of the metencephalon, this portion of the brain integrates motor and sensory information to coordinate planning, timing and patterning of movements and posture. Anatomically it consists of bilateral cerebellar hemispheres and deeper nuclei; we will examine the former.

As shown in your text (Kerr) Figure 6.12 C, the cerebellar hemispheres consist of three cell layers surrounding a tract of white matter. Most superficially and least densely populated is the molecular layer. The granular layer is adjacent to the white matter tracts and is densely populated by the small granule cell neurons. Positioned between the molecular and granular cells layers are the large Purkinje cells.

Due to the thickness of the silver stained specimen (93W3623) and the capriciousness of silver staining, these layers and cells may be difficult to observe in this slide and you may wish to examine the H&E stained specimen at the demonstration table first.

Identify:

- molecular cell layer
- Purkinje cell layer (Again, ignore Dr. Kos' pronunciation)
  - Purkinje cells
- granular cell layer
  - granule cells
- white matter (tracts)

FYI: In humans, there are as many neurons in the granular cell layer of the cerebellum as in the rest of the brain combined.

### III. Cerebral cortex

- cerebrum, silver (Golgi) stain: 93W3620
- cerebrum, Nissl (cresyl violet) stain: DEMONSTRATION

We conclude our study of the CNS with an examination of cerebral cortex. Unfortunately the silver stained specimen appears to be an incomplete cross-section and thus the classical 6 cell layers (see Kerr Figure 6.7) do not appear to be preserved. However, the various types of cortical neurons can be distinguished. Pyramidal cells are the dominant type (up to 70%) and as their names suggest their somas are pyramidal in shape. Stellate cells are smaller and multipolar, giving a star-like shape. The Martinotti cells have somas with lots of projections and long ascending axons. The fusiform cells have spindle-shaped somas and are typically located in the deeper layers.

Identify:

- pyramidal cells

### IV. (Peripheral) Nerves [Can you say "redundant"? Sure you can!]

- nerve, cross section, H&E: H 1578, 93W3647
- nerve, cross-section, myelin stain (osmic acid): 93W3643
- nerve, teased fibers in longitudinal section, osmium tetroxide: H1670 (extras available) – good for nodes of Ranvier
- tail: H800 (best); 93W3321 (faded)
- tongue, longitudinal section (H2690; extras available)

For the peripheral nervous system (PNS) the pickings from your slide box in regards to ganglia are slim. Unless you were lucky enough to have a spinal ganglion on the spinal cord slide, we'll be limited to looking at nerves. Start by looking at nerves in cross-section. In H&E

stained paraffin sections the axons are typically poorly stained and surrounded by a thin myelin sheath or anulus. The nuclei associated with the anulus are the Schwann cells. In the osmic-acid stained section, the myelin lipid of the Schwann cells have been preserved, thus giving a better representation of living condition. Note that interspersed between the axons and their myelin sheathes are wisps of connective tissue called the endoneurium. Groups of axons are gathered into fascicles surrounded by a connective tissue perineurium and these fascicles are formed into a nerve by the perineurium.

Schwann cells in the PNS and oligodendrocytes in the CNS form the myelin sheath. This sheath is not continuous and the gaps between adjacent neuroglial cells are called the nodes of Ranvier. These nodes are best seen in the teased fiber preparation. Recall that this spacing of the myelin sheath is responsible for the saltatory conduction of nerve impulses.

Now look for nerves intermixed with other tissues. You should be able to identify nerves in cross-section in the tail and in longitudinal section at the base of the longitudinally sectioned tongue.

**N.B.** In longitudinal sections nerves can be confused with both smooth muscle and collagenous fibers. Nerves have a slightly wavy appearance, a “washed-out,” spongy cytoplasm around the central axons, and a diversity of nucleus shapes (flat for endoneural fibroblasts; rounder, lighter staining Schwann cell). Blood vessels are rare in nerves. In contrast, smooth muscle has a darker, more homogenous cytoplasm and more uniform nuclei, both in shape (fusiform), size and distribution. Collagenous tissue has uneven, undulating, dark staining fibers with uniform staining. The nuclei are uniform, dark staining and flattened. Blood vessels are common.

Identify:

axons  
myelin annulus  
Schwann cells (don't ask Dr. Kos for the pronunciation)

epineurium  
perineurium  
endoneurium

#### V. Neuromuscular junctions

motor nerve endings, silver stain: H4.31 (Turtox; OK); 93W3657 (lovely)  
Acetylcholinesterase (AChE) whole mounts: DEMONSTRATION  
Combined AChE / anti-neurofilament antibody slide: DEMONSTRATION

The largest synapse in vertebrates is the neuromuscular junction formed by the axon terminals of (alpha) motoneurons and skeletal muscle fibers. The silver-stained specimen shows axons breaking into multiple axon terminals, each covering a neuromuscular junction.

The distributions of neuromuscular junctions within a muscle can be demonstrated using acetylcholinesterase histochemistry (this enzyme degrades the synapse's neurotransmitter, acetylcholine). At the demonstration table are examples of this reaction in both whole mounts and sections.

Identify:

axons  
axon terminals  
neuromuscular junction

#### VI. Sensory mechanoreceptor: Pacinian corpuscle

Pacinian corpuscle (pancreas; H&E): 93W3667

Our homage to the sensory component of the nervous system will be brief, one mechanoreceptor and one chemoreceptor. Examine this section of the pancreas and find the rather obvious Pacinian corpuscle (See Kerr Figure 8.11). These large, encapsulated sensory nerve endings are sensitive to deep pressure and vibration and are commonly found in the skin, joints and tendons. Would anyone care to explain their presence in exocrine glands?

Identify:

Pacinian corpuscle

VII. Sensory chemoreceptor: Vomeronasal organ (of Jacobson)

fetal skull / membranous bone (trichrome): 93W3283

The majority of vertebrates (craniates) have two chemoreceptors to detect air-borne chemicals, the olfactory neurons and the vomeronasal organ. The vomeronasal organ (VNO) is located in the palate and chemicals, particularly pheromones, are conveyed to it by the tongue. [Humans, at least adults, appear to lack the VNO.]

In your section of the fetal skull, look for the classic “mushroom bodies” of the VNOx on either side of the longitudinal midline. Histologically, the mucosa of the VNO and olfactory neurons are identical (see Kerr Figure 19.2.). Note this sensory mucosa consists of a pseudostratified columnar epithelium with a ciliated surface. See if you can observe any nerves emanating from the organs.

For a recent article on the importance of pheromones and VNO on behavior, see the article from the February 1, 20002 issue of science at the demonstration table.

Identify:

vomeronasal organ  
cilia

VIII. Questions & Quiz

As always, time has run out so you will be supplied with both questions and a list of structures for the nervous system quiz.