Exercise 5
Nervous Tissue

Objectives

After completing the laboratory exercise for nervous tissue you should be able to:

- **Identify** a "typical" multipolar neuron in the light microscope.
- **Identify** microscopic sections of ganglia and be able to distinguish between craniospinal ganglia and autonomic ganglia.
- **Identify** microscopically the supporting cells of nervous tissue. Identify the different regions of the spinal cord in the light microscope.
- **Identify** microscopic sections of peripheral nerves. Identify a neuromuscular junction in a microscopic preparation.
- **Identify** in microscopic preparations the axon, the myelin sheath, and the Schwann cell sheath. Identify selected receptors such as: a) Pacinian corpuscles; and b) neuromuscular spindles.
- **Identify** the various layers and components of the cerebellum.
- **Identify** the six layers of the cerebral cortex.

Introduction

All nervous tissue consists of nerve cells (neurons), supporting cells (glial cells or neuroglia), and blood vessels. The nervous system is divided into two major parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of neurons and axons located in the brain and the spinal cord; it is the integrating and communicating center of the body. The PNS consist of neurons and axons located outside the CNS. These include cranial nerves from the brain, the spinal nerves from the spinal cord, and both of their associated ganglia. The brain and the spinal cord consist of white matter and gray matter. White matter consists primarily of myelinated axons, some un-myelinated axons, and the supporting cells. Presence of myelin sheaths around the axons imparts a white color to the region. Gray matter is composed of neurons, dendrites, and neuroglial cells. Lack of myelin sheaths imparts a gray color to this area of the CNS.

In this laboratory exercise, you will see slides stained with different stains than the ones that have been used in previous exercises. While H & E is useful as a general stain in most tissues, the high lipid content of nervous tissue makes it less suited to H & E than most others. Consequently, numerous stains have been developed which take advantage of the chemical properties of the lipids in neural tissue to highlight structural features.

One of the most common stains used for nervous tissues is the cresyl blue method, which binds very strongly to the RNA in the neurons rough endoplasmic reticulum since it is a basic stain. Luxol fast blue is another common method which gives particularly good delineation of nerves tracts in the CNS and the two are commonly used together.

Osmium tetroxide is both a stain and a fixative. It is used primarily as a fixative in electron microscopy, but since it binds to lipids quite well, it is particularly well suited to reveal the details of myelin in nerves. When it reacts with the double bonds of lipids, osmium tetroxide is reduced and metallic osmium is deposited in the tissue. This gives the tissue a black coloration, and nerve fibers in particular react strongly.

There are hundreds of other staining routines, much of which involve the use of gold or silver salts. Among the most elegant of these stains are the ones developed by Camillo Golgi (1843 – 1926) or Santiago Ramón y Cajal (1852 – 1934) who shared a Novel prize for their work in 1906. These methods are especially useful for visualizing glial elements.

I. The Basic Unit: The Neuron

A typical neuron is a non-dividing, long lived cell with a conspicuous nucleus surrounded by cytoplasm. The cytoplasm contains characteristic basophilic clumps of RER called Nissl bodies. The many short thread-like processes that extend from the cell body are the dendrites. The neuron has a single axon, sometimes up to a meter long.

Neurons may be classified according to the morphology of the axon and dendrites, or more commonly, according to the number of cells processes extending from the body.
- **Multipolar neurons** — common in the CNS and in the ANS (autonomic nervous system), have one axon and dozens of dendrites.
- **Bipolar neurons** — found in the olfactory epithelium and retina, have only two processes — one axon and one dendrite
- **Pseudounipolar neurons** — (also called unipolar) found in the dorsal root ganglia in the PNS, have a single short process that functions as an axon, and branches as a T-shape, of which one process leads to the spinal cord and the other extends to the peripheral tissue.

List of slides – Neurons

| CNS 1 | Multipolar |
| CNS 2 | Multipolar |
| CNS 3 | Multipolar |
| CNS 4 | Isolated spinal cord, smear |

**REVIEW BOX SLIDE 22**

**Note:** When looking at these slides be sure to study areas in which there are neurons. Most of the other cells in these preparations are neuroglia. Neuroglia cells can be distinguished by their much smaller size and their dark-staining nuclei. These cells outnumber neurons in the nervous system by a factor of 10.

**II. The Central Nervous System**

**A. The Brain**

The brain consists of the **cerebellum**, **cerebrum** and **brain stem**, and each part consists of gray and white matter. Gray matter is composed of neuron cell bodies, neuroglial cells and blood vessels. White matter is composed mainly of myelinated axons and neuroglial cells, and provides routes or nerve tracts that connect one part of the brain to another and connects the brainstem to the spinal cord.

**Cerebellum**

The cerebellum consist of two hemispheres, deeply folded into fissures and lobes, which are composed of an outer covering of gray matter and inner, branched cores of white matter.

The cortex of gray matter consists of three layers:

- **Outer molecular layer** – (mainly synaptic)
- **Middle layer** – of single, large Purkinje cells, with dendrites in the molecular layer and axon passing into the white matter.
- **Inner granular layer** – contains billions of small granule cells, which receive input from the mossy fibers. In turn granule cells send very small axons into the molecular layer. These axons branch to form parallel fibers that make tens of thousands of synapses with the dendritic tree of the Purkinje cells.

List of slides – Cerebellum

| CNS 5 | Cerebellum, human t. s. |
| CNS 6 | Cerebellum, monkey t. s. |
| CNS 11 | Cerebellum and brain stem, rat |

**REVIEW BOX SLIDE 82**

**Cerebrum:**

Over 80% of the brain is cerebrum. The two cerebral hemispheres each consist of the outer folded cortex of gray matter that covers the inner white matter, within which are additional collections of gray matter called basal nuclei and thalamic nuclei.
Histologically the cerebral cortex is most easily studied because the neurons form six layers parallel to the cortical surface. Starting in the periphery of the cortex, the outermost layer is the molecular layer. Its peripheral portion is composed predominantly of horizontally directed neuronal processes, both dendrites and axons. Deep in the molecular layer lie the infrequent stellate or spindle-shaped horizontal cells of Cajal, their axons contribute to the horizontal fibers. Overlying and covering the molecular cell layer is the delicate connective tissue of the brain, the pia mater.

In the next four layers, the predominant cells are the characteristic pyramidal cells of the cerebral cortex, these cells exhibit variable sizes. The pyramidal cells get progressively larger in layers 2, 3, 4, and 5. In the 6th layer, the multiform layer, there are no pyramidal cells; however, the fusiform cells predominate and the granule cells stellate cells and cell of Martinotti are intermixed. All of these cells vary in size.

List of slides – cerebrum

CNS 7 Cerebrum
CNS 10 Cerebral cortex, Nissl
CNS 12 Cerebrum, cerebellum, medulla
CNS 13 Acute meningitis

Neuroglia:

Glial cells are specialized, non-neuronal supporting cells within the CNS found in close association with neurons. Astrocytes (star cells) are the largest neuroglia cells. In the CNS astrocytes are attached to the walls of the capillaries and to neurons. Astrocytes have numerous long, smooth, slightly branched processes extending in all directions.

Other types of glial cells in the CNS include:

- Oligodendrocytes – mostly in CNS white matter
- Microglia – CNS phagocytes which remove cellular debris and damaged cells
- Ependymal cells – line the ventricles as cuboidal or columnar cells

List of slides – Neuroglia

CNS 14 Astrocytes
CNS 15 Glial cells

The Spinal Cord:

At most vertebral levels cross-sections through the spinal cord show the distinctive central butterfly-shaped mass of gray matter surrounded by the ascending and descending tracts of myelinated nerves, and the glial cells of the white matter. In the cross section, you will easily see that the gray matter takes an H shape, with a small channel, the central canal, in the middle. The central canal, like the brain ventricles, is filled with cerebrospinal fluid and lined with ciliated cells that circulate the fluid.

In those slides that are silver stained (Cajal method) the architecture of the neurons and their axons are beautifully defined. TAKE A GOOD LOOK!

List of slides – Spinal cord

CNS 16 Spinal cord, rat
CNS 17 Spinal cord, t. s.
CNS 18 Spinal cord, cat
CNS 19 Spinal cord, silver
CNS 20 Spinal cord, Nissl and myelin
CNS 21 Spinal cord, mammal
CNS 25 Spinal cord, frog
CNS 26 This slide review all tissues

REVIEW BOX SLIDE 83
To view the brain in different groups of animals study the following slides:

- CNS 22  Dogfish
- CNS 23  Amia
- CNS 24  Salvelinus

### III. The Peripheral Nervous System (PNS)

#### Nerves:

Nerves are composed of axons of various sizes and their surrounding sheaths. In the PNS, all axons are surrounded by Schwann cells. The cells extend along the length of a peripheral axon, from its origin to its termination in a muscle or gland. Smaller axons are surrounded only by the Schwann cell cytoplasm. Such axons do not have a myelin sheath and are un-myelinated. Larger axons are wrapped by successive layers of the plasma membrane of the Schwann cell, which produces the insulating myelin sheath.

In histologic sections stained with hematoxylin and eosin (H & E), peripheral nerves are poorly stained, and to the untrained eye, may go unnoticed or be identified as connective tissue. As myelin is mostly lipid, the sheaths are dissolved during the tissue preparation process, which leaves empty spaces. Depending on the quality of specimen preservation and intensity of H & E staining, the characteristic features of nerves can be recognized, but the use of specific fixatives (such as osmium tetroxide) and various connective tissue stains (such as Mallory, Masson, or Van Gieson) greatly improves the structural detail.

Larger nerves are bound by a complete outer layer of dense connective tissue, called **epineurium**, below which we can find loose connective tissue with blood vessels and variable quantities of fat. Axons are grouped together to form bundles, or fascicles, and each fascicle is surrounded by another connective tissue sheath called **perineurium**. Individual axons, together with their Schwann cells, are supported by thin, branched layers of **endoneurium**, through which capillaries supply the neural elements.

#### List of slides – Nerves

- PNS 1  Myelinated fibers, teased
- PNS 2  Myelinated fibers, teased
- PNS 3  Non-myelinated fibers, teased
- PNS 4  Nerve bundle, c. s.
- PNS 5  Nerve bundle, c. s. and l. s.
- PNS 6  Artery, vein, nerve
- PNS 8  Vascular bundle
- PNS 9  Neurovascular bundle, monkey, t. s.
- PNS 10  Muscle-nerve, human, t. s.

#### REVIEW BOX SLIDE 23

#### Ganglia

- **Sensory (dorsal root, spinal) ganglia** — are swellings of the dorsal roots of spinal nerves and contain pseudo-unipolar neurons clustered together and surrounded by numerous glial cells. Each cell body has one process resembling an axon, which bifurcates within the ganglion, one branch extending peripherally to its site of origin, the other acting as an efferent branch and traveling to the gray matter in the spinal cord.

- **Autonomic ganglia** — are associated with the sympathetic and parasympathetic divisions of the ANS. In structure, autonomic ganglia are similar to sensory ganglia except that the cell bodies are dispersed and fewer glial cells are present as these neurons are multipolar, their radiating dendrites snapping with motor signals transmitted by the pre-ganglionic fibers that originate in the CNS.

#### List of slides – Ganglia

- PNS 11  Spinal ganglion
- PNS 12  Sympathetic ganglion
Motor end plates

The motor end plates of skeletal muscle have the same basic structure as other synapses. In motor end plates axons terminate at the sarcolemma, and excitatory nerve impulses initiate electrical impulses in the sarcolemma via release of acetylcholine from axon synaptic vesicles.

List of slides – Motor end plates

| PNS 13 | Motor end plates |
| PNS 14 | Motor nerve endings |

REVIEW BOX SLIDE 25

Sensory receptors

Sensory receptors are nerve endings or specialized cells which convert stimuli from the external or internal environment into afferent nerve impulses. The impulses pass into the CNS where they initiate voluntary or involuntary responses.

Functional classification divides sensory receptors into three groups:

- **exteroreceptors** – respond to stimuli from outside the body; include receptors for touch, pressure, pain, temperature
- **proprioceptors** – located with the skeletal system and provide conscious and unconscious information about orientation, skeletal position tension and movement
- **interoreceptors** – respond to stimuli from the viscera

Sensory receptors may be classified morphologically into two groups: simple and compound. Simple receptors are free, branched or un-branched nerve endings such as those responsible for skin pain and temperature. Compound receptors involve associated non-neural tissues to complement the neural receptors. Degree of organization may range from mere encapsulation to highly sophisticated arrangements such as the eye and ear. By tradition the eye, ear and receptors for the senses of smell and taste will be covered at a later date.

Lists of slides – Sensory receptors

| PNS 15 | Pacinian corpuscles |
| PNS 16 | Pacinian corpuscles |
| PNS 17 | Vater-Pacinian corpuscle |
| PNS 18 | Pacinian corpuscles |
| PNS 19 | Meissner’s corpuscle |
| PNS 20 | Meissner’s corpuscle |
| PNS 21 | Muscle spindle |
| PNS 22 | Tendon spindle |

REVIEW BOX SLIDES 84, 86

Additional information

- **Meissner’s corpuscles** – are small, encapsulated receptors found in the dermis of the skin. They are involved in the reception of a light discriminatory touch. They are oval in shape and consist of a collagenous capsule surrounding a mass of plump, oval cells arranged transversely. Branches of sensory fibers ramify throughout the cell mass.
- **Pacinian corpuscles** – are large encapsulated sensory receptors responsive to pressure or coarse touch, vibration and tension. They are found in the deeper layers of the skin, ligaments and some viscera. These organs consist of a delicate capsule enclosing concentric lamellae of flattened cells. The core of the corpuscle contains a single, large un-branched non-myelinated nerve fiber which becomes myelinated as it leaves the corpuscle.
- **Muscle spindles** – are stretch receptors within skeletal muscles responsible for the regulation of muscle tone. Spindles are encapsulated, lymph filled, fusiform structures that lie embedded in endomysium or perimysium. Each spindle contains a number of modified skeletal muscle fibers. Nerve endings are associated with the organ.