Student guide - Neurohistology

HM591 – fall 2018 – Dr. Lovell

Objectives (T=Topic):

Part A – PNS - Understand the appearance and variability of different types of cells in the PNS.
T1: Dorsal root ganglion
T2-3: Peripheral nerves and Schwann cells
T4: Neuromuscular junction

Part B – CNS - Understand the appearance and variability of different types of cells in the CNS
T5: Ventral horn motoneurons,
T6: Cerebral cortex
T7: Substantia nigra
T8: Structure of synapse
T9: Astrocytes – relationship to synapses and blood vessels

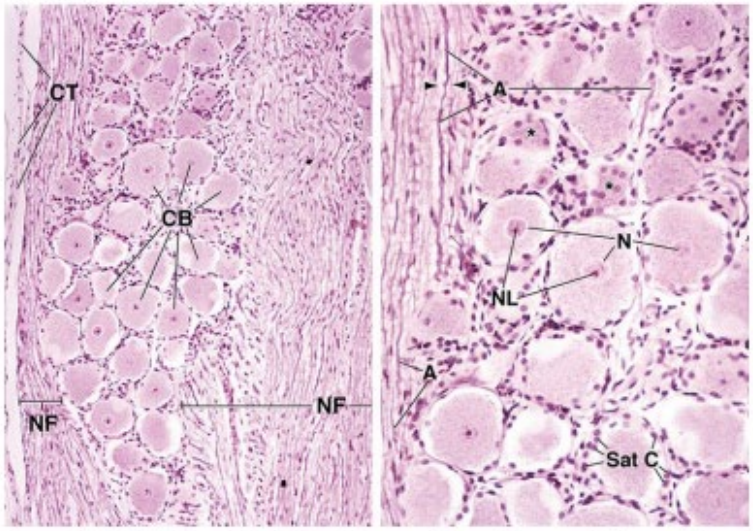
Part C – Blood-Brain Barrier (BBB) and fMRI -Understand the components and function of the BBB
T10: Astrocyte foot processes, endothelial cell tight junctions, pericytes, basal lamina; neurovascular unit

Topic 1: Posterior (dorsal) root ganglion in LM (H&E stain)

All somatic or autonomic ganglia are clusters of cell bodies located in the PNS (outside of the meninges covering the CNS). Images below are centered on the posterior (dorsal) root ganglion of this section through the spinal cord.

- **Find neuron somas (neuronal cell bodies) and their satellite cells.** The neuron somas of the posterior root ganglion are fairly uniformly round (spherical in 3D). Satellite cells in this type of ganglion form a well ordered ring around each neuron soma section, but would completely surround the soma in 3D.
- Note the axons that travel through the ganglion. Each axon arises as a single process from the soma of a neuron. The sites of origin of the axons in the posterior root ganglion are seldom included in the section, because they are a single small point on the surface of the very large somas.

What is the function of neurons in the posterior root ganglia?
Why are the neuron cell bodies so large?



Pawlina Plate 21 Chp 12: CT= Connective Tissue; CB= cell body of neuron; NF = nerve fibers; A = Axon; N = nucleolus; Sat C = Satellite Cell (a neuroglial cell)

Topic 2: Peripheral nerve in cross section: axons, Schwann cells, connective tissue (osmium tetroxide stain). Osmium tetroxide, when used as a fixative, binds to the phospholipid components of membranes, such as myelin which is darkly stained.

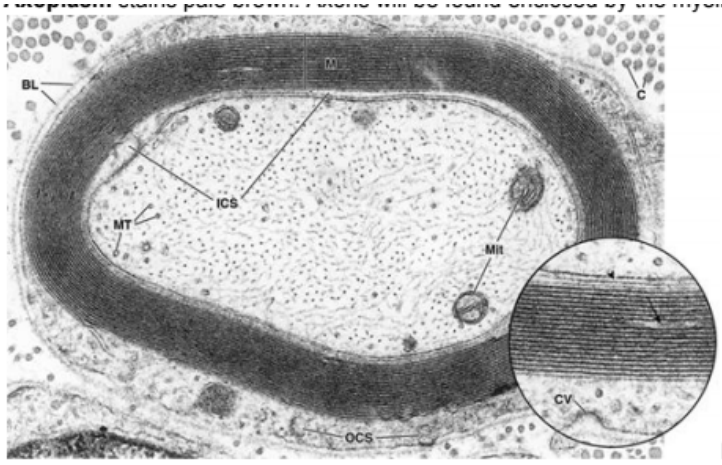
In these images you can see nerve fibers arranged in bundles, which are round in cross section. Each of these bundles is one fascicle, a collection of axons. The sizes of fascicles vary. Nuclei within the fascicle are those of the Schwann cells.

- Epineurium is connective tissue between fascicles and around groups of fascicles.
- Perineurium is connective tissue immediately surrounding each fascicle.
- Endoneurium is connective tissue around and between individual nerve fibers and external to their associated Schwann cells. Therefore, when examining myelinated nerve fibers, the endoneurium is identified external to the myelin sheath which encloses individual nerve fibers. In unmyelinated nerve fibers, the endoneurium is identified external to the Schwann cell that encloses a group of axons.

The diameter of axons is variable. Unmyelinated axons are smaller in diameter than are myelinated axons.

What is the relationship between diameter and the propagation velocity of an action potential in an axon?

Identify fascicles, epineurium, perineurium and endoneurium.
Identify myelin sheaths, myelinated and unmyelinated axons, axoplasm, Schwann cell nuclei.



Pawlina 12.12

This Pawlina Figure 12-12 shows a 19 layer myelin sheath (M) from a single Schwann cell. The axoplasm (cytoplasm of axon) demonstrates microtubules (MT) and mitochondria (Mit). Collagen fibrils (C) of the endoneurium are shown in the upper right and upper left corners and is outside of the Basal Lamina (BL) the immediately surrounds the Schwann cell. Ignore remaining acronyms in figure.

Topic 3: Peripheral nerve in longitudinal section (toluidine blue)

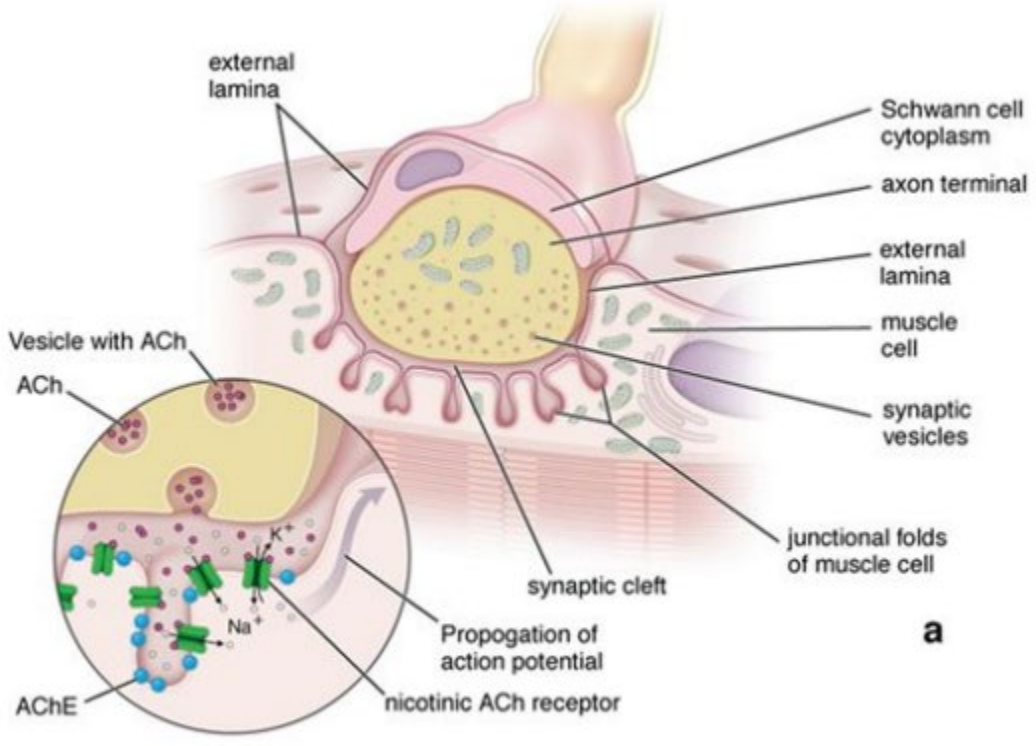
These images are of a longitudinal section of a nerve. Unsing this toluidine blue stain, axoplasm is pale blue and the myelin sheaths are dark blue. Between myelin sheaths you will see pale blue endoneurium. Within the fascicles you will see axons cut in longitudinal, oblique and roughly cross section.

Identify nodes of Ranvier.
Which types of channels are at high density at nodes of Ranvier? How do they contribute to action potentials?
Describe the components in the electron microscope image in Image 5.

Topic 4: Neuromuscular junction in LM, whole mount preparation.

In this preparation, cylinders of skeletal muscle fibers were teased apart and laid flat with a fine needle to expose the terminal branches of several somatic motor neurons. The individual skeletal muscle fibers are stained with H&E. An additional stain highlights the axons in black as they extend to the motor end organs. These images follow axons to their termini on the skeletal muscle fibers. Examine the terminus of an axon, which forms a rosette of small, round endings. Each of these endings corresponds to a single neuromuscular junction (NMJ) which is the synapse between the terve terminal and the motor end-plate region of the skeletal muscle fiber. There is much variation in the numbers of termini in each rosette, depending on the type of muscle and size/type of motor neuron. The diagram below is a reminder of the components of a NMJ.

Identify a cluster of NMJ terminals from a single axon.
What neurotransmitter is used? How is the action of this transmitter terminated? What type of receptors are embedded in the postsynaptic membrane?

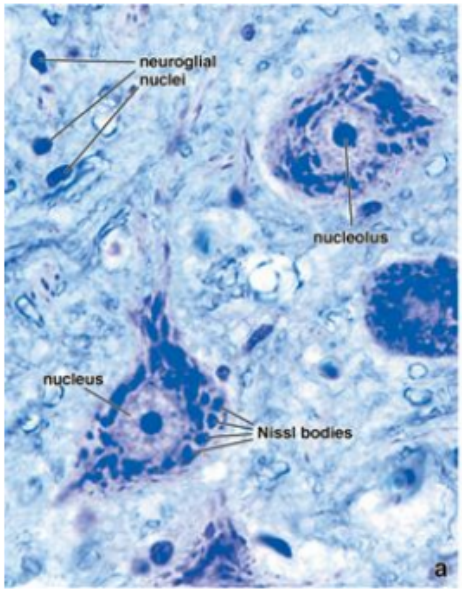


Pawlina 11.4 shows a single NMJ

Topic 5: Spinal cord motor neurons (LMNs) in ventral (anterior) horn

Spinal cord neurons, axons, soma in LM with Cresyl Violet (Nissl) stain & Luxol Fast Blue Stain

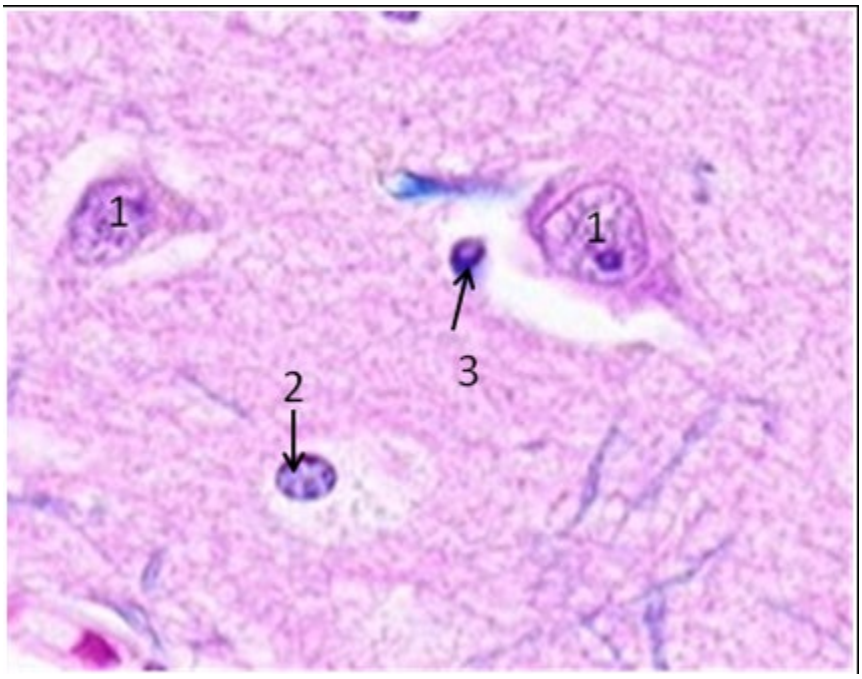
- **Cresyl Violet (Nissl) stains** bind to DNA and RNA. Neurons commonly have large amounts of RER, and this stain is used to visualize the clumps of RER in the cytoplasm. These clumps, or **Nissl bodies**, will be intensely stained with Nissl stain, as will the nucleic acids of the nucleus and nucleolus.
- **Luxol Fast Blue** (LFB) stain stains CNS myelin well and is used to evaluate CNS demyelination, but cannot evaluate PNS myelination.
- The gray matter of this spinal cord section forms roughly an "H", or butterfly shape, in the center of the spinal cord. The posterior (dorsal) surface of the spinal cord is at the top of this section.
- These images will center on the anterior (ventral) horns of gray matter. Here you will see large neuronal **cell bodies**. The neuronal cell bodies in this area belong to somatic motor neurons (a.k.a. general somatic efferent neurons)
- Notice the large, pale nuclei and prominent nucleoli of these cells. Outside the nucleus are blue-to-purple clumps of material within the cytoplasm. These are **Nissl bodies (Nissl substance)**, which represent the rough endoplasmic reticulum of these cells. ***Based upon your previous studies, what do these histologic characteristics tell you about the activity of these cells?***
- Note the projections emerging from the neuronal somas. These are multipolar cells, and the projections correspond to the axon and multiple dendrites emerging from the neuron soma. The blue lines that crisscross among the neuronal cell bodies in the anterior horns of the gray matter are myelinated axons. **Myelin stains blue with the LFB stain.**
- The smaller nuclei between neuronal somas belong to glial cells. Special staining is necessary to highlight the thin cytoplasmic processes of glial cells.



From Pawlinad 12-4 Photomicrograph of spinal cord anterior horn. Nerve cell bodies are large, spherical with pale-staining nuclei and prominent nucleolus and numerous abundant Nissl bodies in cytoplasm. Smaller staining nuclei are neuroglial cells. Remainder of light blue and pale blue structures in field of view are other CNS neurons in anterior horn.

Topic 6: Neurons and glia in the cerebral cortex (H&E stain)

In a routine H&E stain of CNS tissue from the cerebral cortex (and many other regions), neuron cell bodies can be identified by a large nucleus and nucleolus within the soma, along with cytoplasm. For glia, only astrocyte and oligodendrocyte nuclei are seen in normal tissue. Oligodendrocyte nuclei are generally smaller and more darkly stained than astrocyte nuclei. Note that in this section the oligodendrocytes are present in the gray matter, and have functions other than providing myelin sheaths.



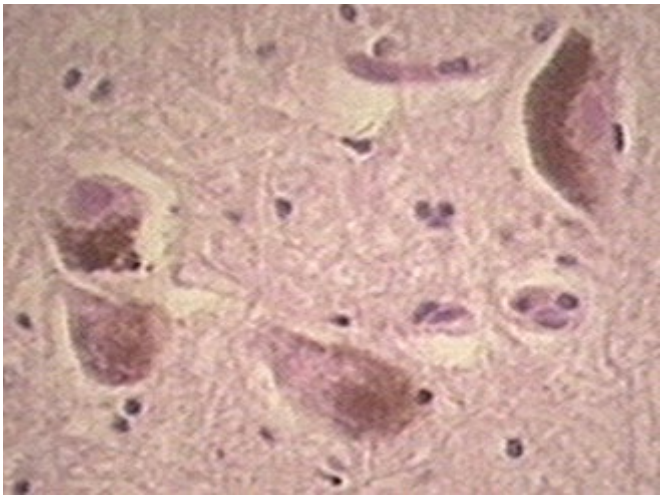
Identify cells labelled 1, 2, and 3.
What type of cell is shown labelled by green fluorescent protein?

Topic 7: Substantia nigra neurons

The top image shows the location of the substantia nigra in the midbrain.

What is the cavity shown?
Identify the tectum and tegmentum.
What transmitter is synthesized in substantia nigra neurons?
Does the synthesis occur in the cell body or axonal terminal? Where are the axonal terminals located?

In the image below, why does the cytoplasm of these neurons contain brown pigment?



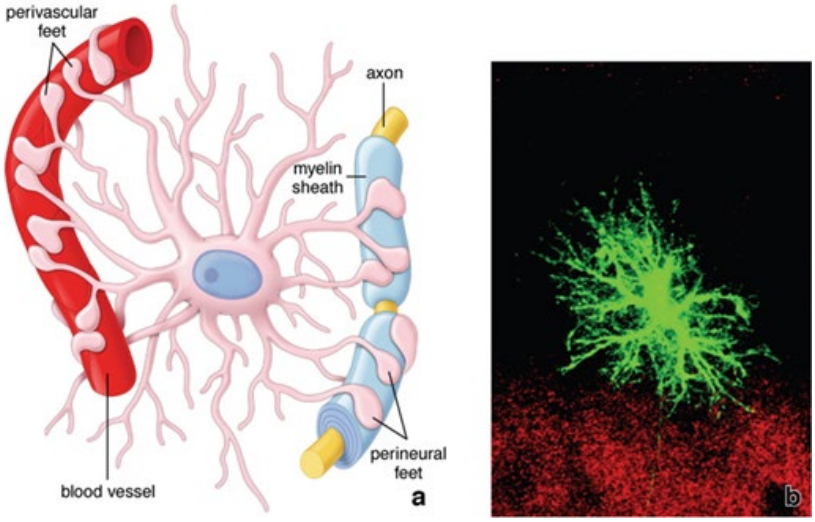
Topic 8: Synapses

Using the diagram of the synapse at top on the screen, identify presynaptic terminal, synaptic cleft, postsynaptic terminal, synaptic vesicles, receptors.
Identify ionotropic and metabotropic receptors in the diagram.
For the synapse illustrated, is a EPSP or IPSP generated at the postsynaptic membrane?
List the main steps in synaptic transmission.

In the electron micrograph, identify presynaptic terminal, synaptic cleft, postsynaptic terminal, synaptic vesicles.

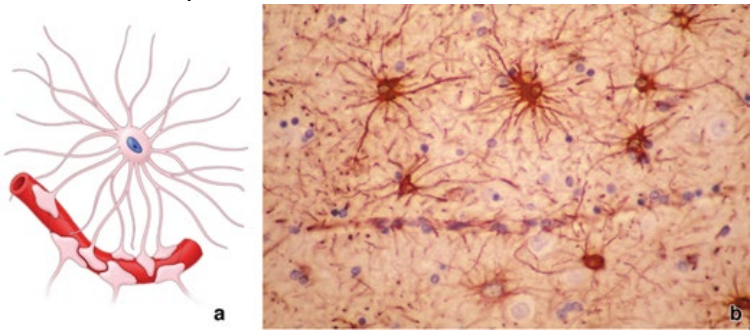
Topic 9: Astrocytes

Protoplasmic astrocyte in the gray matter of the brain.



a. This schematic drawing shows the foot processes of the protoplasmic astrocyte terminating on a blood vessel and the axonal process of a nerve cell. The foot processes terminating on the blood vessel contribute to the blood–brain barrier. The bare regions of the vessel as shown in the drawing would be covered by processes of neighboring astrocytes, thus forming the overall barrier. b. This laser-scanning confocal image of protoplasmic astrocyte in the gray matter of the dentate gyrus was visualized by intracellular labeling method. In lightly fixed tissue slices, selected astrocytes were impaled and iontophoretically injected with fluorescent dye (Alexa Fluor 568) using pulses of negative current. Note the density and spatial distribution of cell processes. ×480.

Fibrous astrocytes in the white matter of the brain



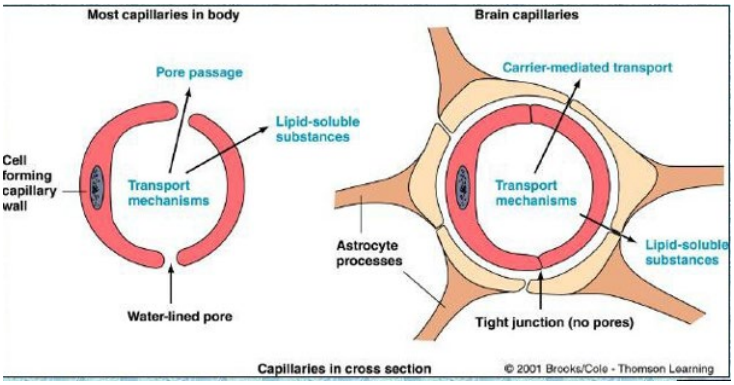
a. Schematic drawing of a fibrous astrocyte in the white matter of the brain. b. Photomicrograph of the white matter of the brain, showing the extensive radiating cytoplasmic processes for which astrocytes are named. They are best visualized, as shown here, with immunostaining methods that use antibodies against GFAP. ×220.
Pawlina Fig 12.18

Both types of astrocytes contain prominent bundles of intermediate filaments composed of glial fibrillary acidic protein (GFAP). The filaments are much more numerous in the fibrous astrocytes, however, hence the name.

Astrocytes play important roles in the movement of metabolites and wastes to and from neurons. They help maintain the tight junctions of the capillaries that form the blood–brain barrier (see page 388). In addition, astrocytes provide a covering for the "bare areas" of myelinated axons—for example, at the nodes of Ranvier and at synapses. They may confine neurotransmitters to the synaptic cleft and remove excess neurotransmitters by pinocytosis. Protoplasmic astrocytes on the brain and spinal cord surfaces extend their processes (subpial feet) to the basal lamina of the pia mater. It is now generally accepted that astrocytes regulate K+ concentrations in the brain's extracellular compartment, thus maintaining the microenvironment and modulating activities of the neurons.

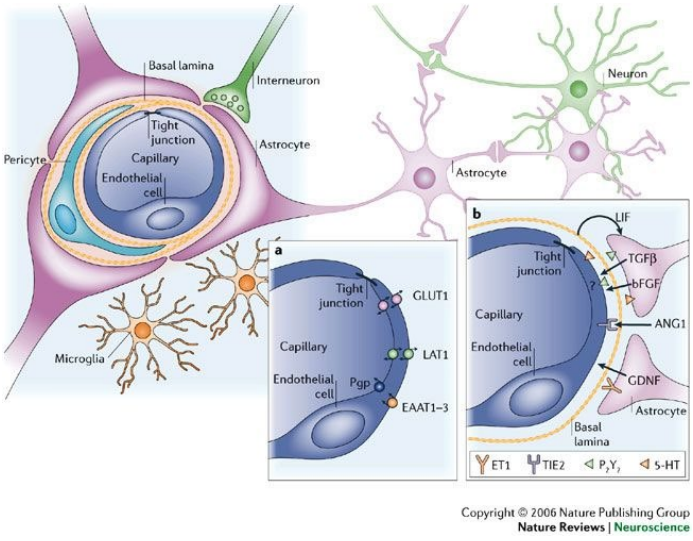
List some of the roles played by astrocyte processes.
What is the function of astrocyte end-feet that surround capillaries?

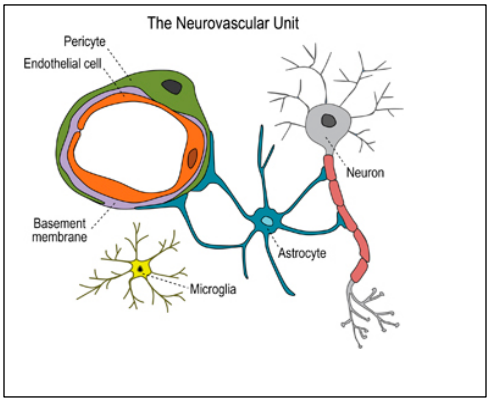
Topic 10: Blood brain barrier



What is the difference between endothelial cells in most of the body compared to endothelial cells in the brain?
What are the components of the blood-brain barrier?
How do glucose, amino acids, and oxygen get from the blood to neurons? What membranes or barriers do they cross?

Functional magnetic resonance imaging, or fMRI, works by detecting the changes in blood oxygenation and flow that occur in response to neural activity – when a brain area is more active it consumes more oxygen and to meet this increased demand blood flow increases to the active area. FMRI can be used to produce activation maps showing which parts of the brain are involved in a particular mental process. This means the blood oxygenation actually increases following neural activation. The blood flow peaks after around 6 seconds and then falls back to baseline, often accompanied by a "post-stimulus undershoot".





Describe the function of the neurovascular unit. How can neuronal activity be visualized using MRI?