Module:
Techniques in Neuroscience

Week 3
Immunohistochemistry: Preserving and studying cells of the brain

Topic 1
An introduction to immunohistochemistry – Part 3 of 4

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As you have already learned, microscopic examination of tissues requires a cutting of thin sections from the tissue so that light can pass through the section. However, excepting where sections are cut from tissues that have been impregnated using the Golgi method, for example, as will be described shortly, the components of the tissue are mostly invisible, so many techniques have been devised that use dyes, heavy metals, or fluorochromes to induce colour or contrast in these components. Additionally, optical contrast of sections can be enhanced using specialist optical techniques, such as phase contrast and differential interference contrast microscopy. But, these are most useful for studying living cells in tissue culture and will not be considered further here. Antibodies raised against specific components of tissues or cells can be used to specifically identify and locate such components, for example proteins, in sections, and these can then be visualised by immunostaining techniques which will be discussed in part 4. However, here in part 3, I will discuss dye staining and metal impregnation techniques. Dye stains are some of the oldest histological techniques available to scientists. Long ago researchers discovered that with specific formulations, different dyes have strong affinities to particular tissue or cellular components. It is important to remember that these dye staining techniques, unlike the immunotechniques that will be discussed later, are selective but not specific. For example, stains such as crystal violet can be used to visualise the presence of Nissl substance that is found in neurons. Therefore, this stain will react with all neurons in the brain section making it an excellent choice for studying the cellular patterns within the specific brain area. However, this technique is not a good choice for studying the detailed morphology of a neuron, or specific types of neurons.

Different histological techniques can be used to investigate specific features of neural structure and function. Shown, here, is a typical neuron, with an axon that is myelinated. The axon is a long process that you can see extending from the neuronal body towards the bottom of the slide and it is surrounded by a substance that insulates and protects it. This substance is called myelin and it is made by cells called oligodendrocytes, a type of glial cells in the central nervous system. This myelin sheath allows electric impulses to travel rapidly along the axon. Let us explore how histology can be used to visualise cells and their processes in the nervous system using a neuron as an example.

Nissl staining is an example of dye staining achieved mainly by ionic interaction between the dye and the tissue component. The Nissl staining method was developed by the German pathologist Franz Nissl at the end of the 19th century and is still widely used today to identify neurons. Neuronal function requires the continuous synthesis of large quantities of proteins such as neurotransmitters. These are the chemical messengers that enables signals to be transmitted from one neuron to another neuron across the synapse, the small gap between the neurons. Neuronal cell bodies house the machinery for protein production called the rough endoplasmic reticulum, or RER. Attached to the RER are ribosomes, the structures where RNA is bound and used as template to assemble many proteins needed by the cell. The RER forms large granular bodies and RNA in these granules can be visualised with basic aniline dyes for example crystal violet. Aniline dyes are synthetic dyes originally made from the analyne that can be obtained from cold tar. Basic or cationic dyes in aqueous solution can ionize to form net positively charged dye molecules or cations. That will combine with net negatively charged components in the cell. That is acidic components or anions. In particular, DNA and RNA. In neurons, because they are actively synthesizing many proteins, aniline dyes but also stain Nissl bodies that are rich with RNA molecules. These will stain more intensely the other cationic components that have lower densities of charge. In the image, note that the nuclei of the two neurons are very pale staining. This is because the DNA is enwound to allow RNA to be transcribed or made. On the other hand, the cytoplasm of the neuron is intensely stained, because this is where the RER is found. The other less intensely stained components are nuclei of other cells that are not so actively synthesizing proteins. In addition, axons and fine structures such as small dendrites will not be visible with this stain. As an aside, the terms acidic and basic are histological terms that refer to the negative or positive charge of a dye in a clear solution. Anionic or cationic will perhaps be better terms to use.

Let me give you two examples. You don't have to know but either of these studies in any detail. They are just being used to give you a flavour of how Nissl staining can be used for study of neural lost following injury or disease. Let's first look at the set images on the left hand side. The images in the bottom panels are from an ischemic gerbil brain, where you can see a much lighter purple stain, indicated by black arrows, when compared to the images in the panels above, which are from a healthy brain. This indicates the loss of hippocampal CA1 neurons, following ischemic damage. Meaning damage caused by the restriction in blood supply to tissue. The images on the right-hand side show you how abnormal growth and development of the cerebral cortex is easily detected with Nissl stain in a mouse model of lissencephaly, a disease caused by defective neuronal migration, resulting in lack of development of the brains grooves, and folds, or sulci, and gyri.

We can also identify cell types other than neurons in brain sections using dyes. Luxol Fast Blue is an acidic dye in solution that is used to visualise central nervous system myelin sheaths in paraffin wax sections. With this technique myelin is stained a deep blue. Remember myelin sheaths as I mentioned earlier are formed by oligodendrocytes in the central nervous system. Composed of cholesterol and glycoproteins, myelin serves to insulate the axons of neurons, which significantly increases the conduction velocity of action potential. This Luxol Fast Blue technique, maybe used to study loss of myelin in neurodegenerative diseases such as multiple sclerosis. Two examples of Luxol Fast Blue staining in the central nervous system are shown on this slide. Both are counterstained with cresyl violet. A counterstain is so called because it either produces a contrasting background colour to the main stain or provides complementary information. In this instance the cresyl violet provides additional information by identify Nissl granules. The upper image shows a ventral hole of a spinal cord where the white matter marinated myelinated tracks run on the periphery in transverse section. Note the purple staining of the Nissl in multi neurons due to the cresyl violet counter stain. The lower image shows intense blue staining of the myelin in the white mater in cerebellum in longtitudinal section. The nuclear cells of the granule cell are pale blue due to cresyl violet counter stain.

So the Luxol Fast Blue stain is useful in a study of myelinated nerve tracts in the central nervous system, and changes that may occur to myelination. Here are two examples of studies where the staining has been used.

Again you do not have to know details of the studies. On the left hand side are images from a study by Lovato et al, Luxol Fast Blue and cresyl violet stains, we use to effectively demonstrate complete loss of myelinated axons of a corpus callosum shown by the black arrow. And malformation of the hippocampus, shown in the box inset, in mice lacking the tumor suppressor neurofibromatosis two gene, which has a role in brain development. By combining the two stains we can visualise both the neuron of cells bodies and their myelinated axons, visualising the structure of the entire brain. The images on the right hand side demonstrate how Luxol fast blue may be used to detect loss of myelin due to injury. Here we can see the complete loss of myelin in the white matter of the mouse spinal cord after contusion. Contusion occurs due to traumatic injury and often causes inflammation and bleeding from blood vessels near the injury site.

There are also staining methods that allow visualisation of fine structures such as dendrites and even dendritic spines. Remember that neurons receive information from other neurons via the dendritic spines that protrude from the dendrites and pass on this information via their axon. The Golgi stain, not really a stain, but a metal precipitation, was developed by Camillo Golgi, and first published in 1783. It was modified by Santiago Ramon y Cajal to draw neurons and neuronal circuits for the first time, founding the study of neuroscience. Basically a small piece of formalin fixed tissue is immersed in potassium chromate, then silver nitrate. The Golgi methods stained the small, random subsets of neurons in exquisite detail. Also the technique produces little background staining and the neurons are stained in dark black or brown producing a high contrast of fine structures such as dendritic spines. This colour is produced by silver salt deposits in the neuron. However, exactly why only some neurons react is unknown. To illustrate this technique, in the top image you see a drawing of rodent hippocampus made by Ramon y Cajal, using the Golgi method to illustrate neuronal network. The lower image is of a Golgi stained cerebellum section. Part of the Purkinje cell is shown and one can easily identify primary and secondary processes and also the dendritic spines.

Now, some examples. The Golgi stain is often used to study neuronal morphology. The method can be used on very thick sections of the brain, up to 500 micrometers, or even whole brains. This means that for some neuron types, we can visualise the entire neuron and most of their processes in the same section. We can reconstruct the neuron and see what affects, for example, a neurodegenerative disease may have on its morphology. This image on the left hand side is from a paper by Milatovic et al and it shows how this method can be used to stain sections of the mouse striatum from which tracings of medium spiny neurons can be generated. The Golgi stain is also often used to quantify the number of dendritic spines that a neuron has. For example, the image on the right hand side is from a study by Glantz and Lewis, who use the Golgi method to show a loss of dendritic spines on the prefrontal pyramidal neurons with patients with schizophrenia.

Finally, I would like to finish this section by providing you with the list of other histological techniques that you may care to read about.