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Histological Staining of Neural Tissue

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Abstract

In order to examine the cellular, structural and molecular layout of tissues and organs, researchers use a method known as histological staining. In this technique, a tissue of interest is preserved using chemical fixatives and sectioned, or cut into very thin slices. A variety of staining techniques are then applied to provide contrast to the visually uniform sections. In the study of neuroanatomy, histological techniques are frequently applied to visualize and study nervous system tissue.

This video focuses on histological staining techniques for neural tissue. An overview of common brain stains is provided, including those that specifically mark neuronal cell bodies, like Nissl stains, and those that selectively highlight myelinated axons, like the Luxol Fast blue stain. Immunohistological techniques, which take advantage of the specific interaction between antibodies and unique cellular proteins, are also discussed. Next, the preparation of brain samples for staining is described, including the basic steps for fixation, embedding, sectioning, and rehydration of the tissue. The presentation also provides a step-by-step procedure for immunohistological staining followed by a Nissl stain, in addition to practical applications of these techniques.

Transcript

Slices, or sections, of brain tissue are a rich material for studying the structure and function of the brain. However, untreated brain is a visually uniform tissue, like a blank canvas. Staining is a way of “painting” the brain to clearly visualize the cellular, structural, and molecular components of the organ. While most stains share common histological methods, each approach has a unique morphological target. This video will provide an outline of the general principles of brain histology, demonstrate some common staining techniques, and review a few applications of these methods in neuroscience labs today.

Before discussing how neurological staining procedures are carried out, lets review the goals of these techniques.

Histological stains are commonly used to provide contrast, thereby revealing features that cannot be distinguished in unstained tissue. For instance, to visualize the neuron cell bodies, or somata, that make up the gray matter of the nervous system, multiple stains are available. The dyes used in Nissl stains attach to nucleic acids, coloring the somata purple and revealing neuronal organization.

Alternatively, to look at white matter, you can stain myelin - the fatty acid sheath surrounding axons - with dyes like Luxol Fast Blue. Alternatively, immunohistochemical staining can highlight molecular targets found on specific cell types. This technique takes advantage of the specificity between antibodies and molecular targets known as antigens. The use of antibodies fused to enzymes or fluorescent compounds allows researchers to visualize their binding sites using enzymatic reactions or fluorescence.

Now that you’ve been introduced to the idea of staining sections, lets look at the common histological steps that precede brain tissue staining and ensure optimal conditions for visualization.

To preserve tissue structure, the brain is first perfused - meaning the blood is drained from the brain and the animal’s vasculature is used to deliver a chemical fixative. After dissection, the brain is fully immersed in fixative to complete the preservation process.

Next, the specimen is embedded in a medium with similar mechanical properties to brain tissue, like paraffin wax. After embedding, the brain is thinly sectioned using an instrument known as a microtome. The slices are then mounted on slides and allowed to dry.

Since most dyes are water-based, the tissue needs to be dewaxed and rehydrated before staining can begin. To accomplish this, the slides are rinsed in xylene to dissolve the wax prior to gradual rehydration through a series of increasingly diluted ethanol. This process should be carried out in a fume hood while wearing the appropriate personal protective equipment.

Having prepared your brain tissue, you are now ready to begin the staining procedure.

The first step, known as blocking, reduces background staining caused by nonspecific antibody binding. Exposing the section to serum accomplishes this by introducing unlabeled antibodies that bind to non-target sites. After a 30-minute to overnight block, the tissue is ready for incubation with primary antibody, which binds to specific molecular targets.

Antibodies are usually diluted in a blocking solution containing detergent, which disrupts cell membranes and allows antibodies to enter the cytoplasm.

After an overnight incubation, excess primary antibodies are removed by brief washes in buffer. Next, the secondary antibodies, conjugated to enzymes or fluorophores, are introduced to specifically label the primary antibodies. Several hours later, excess antibodies are removed with multiple changes of buffer.

Now that the targets have been labeled, let’s talk about how to detect them. For enzyme-conjugated secondaries, this is accomplished by introduction of a chromogenic substrate. When the substrate interacts with the enzyme, a color change occurs, hence the term “chromogenic”. Once the desired staining is achieved, typically after about 2 minutes, the reaction is stopped by quickly immersing the sections in water.

After antibody staining, a second step may still be required to provide some neuroanatomical context to the results. For example, you may choose to enhance tissue contrast using a Nissl stain. This procedure relies upon on basic dyes like Cresyl violet, which interact with nucleic acids.

The first step is to filter the dye solution to remove undissolved crystals. The slides are then incubated in stain until the desired contrast is achieved. Then, wash the sections in multiple changes of water to stop the staining.

The slides are then immersed in a series of alcohol to clear excess stain and dehydrate the slices. After dehydration, the slices are cleared with xylene, which removes the alcohol, and improves imaging since it has light diffraction properties similar to those of the tissue.

To preserve the stained sections for later analysis, coverslip with a permanent mounting medium and let dry overnight.

Now that we have outlined the procedures and goals of staining lets look at some applications.

First off, histological staining is routinely used to visualize individual neurons. In turn, visualization is required for techniques like laser capture microdissection, in which researchers use a laser to isolate specific cells grown on a thin film. Genetic material can be extracted from the stained and dissected cells, allowing researchers to investigate neuron-specific gene expression.

Staining may also be employed to characterize morphological features of individual neurons. In this experiment, immunohistochemistry is performed on cells expressing GFP to visualize synapse-forming dendrites. Staining is compared between normal and activated cells, revealing that dendrites undergo multiple changes in response to activating stimuli.

Finally, thanks to the specificity of IHC, the expression of individual proteins can be used to identify the location and identity of unique cell populations in the nervous system. For example, staining of the mouse cerebellum with zebrin 2 antibodies reveals the location of specialized neurons known as Purkinje cells.

You've just watched JoVE's introduction to targeted staining of brain slices. In this video we have demonstrated the general methods and goals of staining, in addition to specific procedures for IHC and Nissl staining.

Thanks for watching!