KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Carl Hobbs

Voice over by Dr Brenda Williams

Module:
Techniques in Neuroscience

Week 3:
Immunohistochemistry: Preserving and studying cells of the brain

Topic 1:
An introduction to immunohistochemistry
Part 1 of 4

Topic list

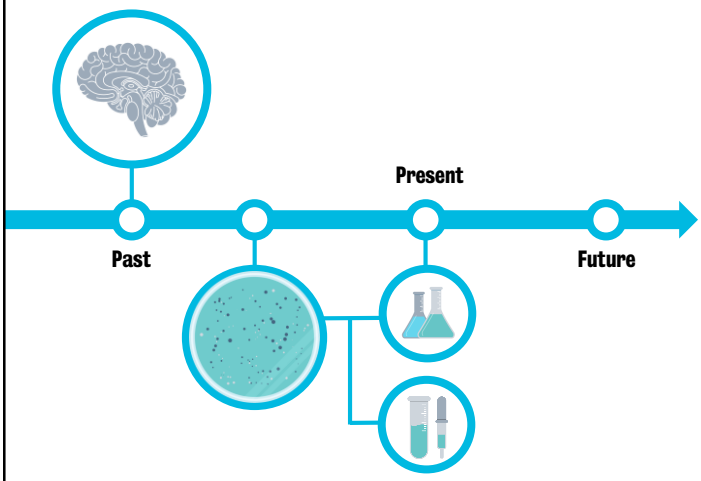
This week, we will be looking at the following topics:

- ***Topic 1: An introduction to immunohistochemistry***
- Topic 2: Video of procedures
- Topic 3: Focused journal club

Click **Next** to continue

Welcome

Histological techniques in neurosciences



- methods of tissue and cell preparation
- principles of histologic stains, metal impregnation and immunohistochemistry techniques
- examples of how these methods are used in neuroscience research

Lecture summary

1 Sample preparation → **2** Basic histologic stains → **3** Metal impregnation → **4** Immunostaining

Part 1

Why examine tissues and cells?

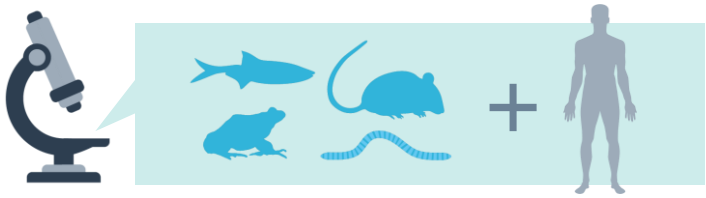
We can study:

- tissue anatomy and cytoarchitecture
- distribution of proteins
- pathological changes associated with disease

Histological study of tissues is essential in:

- clinical diagnostic neuropathology
- basic and translational neuroscience research

Tissue sources



	Animal models	Post-mortem donor tissue, pathology samples, surgical surplus
Advantages	can study different stages of disease	reduce need for animal research
	can study effects of specific mutations	arguably better for studying human disease
	can assess therapeutic strategies	
Limitations	may not fully recapitulate human disease	ethical concerns
	ethical concerns	limited tissue supply
		low availability of early stages of disease

Tissue preparation for histology

Aims:

- to preserve the tissue in as life-like a manner as possible
- to prevent irreversible cell/tissue destruction.

Common methods of tissue preservation:

chemical fixation

cryopreservation

Chemical fixation

Types of fixatives:
acetic acid, formaldehyde, ethanol, glutaraldehyde, methanol and picric acid

Best morphology
Poor staining

Glutaraldehyde

Formaldehyde

Poor morphology
Best staining

Acetic acid, methanol

Chemical fixation process

Chemical fixatives stabilise proteins and other macromolecules.

Cross linking fixatives:

- **formaldehyde and glutaraldehyde**: create covalent bonds between proteins in the tissue
- **aldehydes**: anchor proteins

Protein tertiary structure: the three-dimensional structure of a protein

Precipitating fixatives:

- **ethanol and methanol**: disrupt hydrophobic bonds between proteins causing them to irreversibly precipitate

Hydrophobic bonds: these arise from the interaction of their hydrophobic (water-disliking) amino acids with water.

Methods of tissue fixation

Having taken fresh tissue, it must then be fixed.

Immersion fixation:
fresh tissue is placed in fixing fluid and gently agitated.

Perfusion fixation:
injection of fixing fluid into the circulatory system.

Factors affecting quality of fixation

changes in pH

length of incubation in fixative

specimen size

temperature

Cryopreservation

Preservation of tissue structure and components by freezing them rapidly without fixation.

Advantages:
- fastest method
- minimal changes to protein structure
- rapid cooling (-70°C using liquid nitrogen) minimises damage to the tissue (ice-crystal artefact)

Disadvantages:
- relatively poor morphology
- degradation continues over time
- requires specialist cold storage equipment

Methods:

Snap-freezing the sample with **dry ice** or **liquid nitrogen**

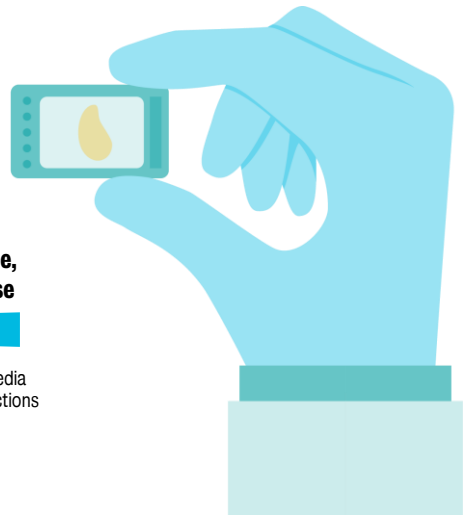
Embedding

Aim:
embedding samples in a solid medium will give support for tissue structure, providing sufficient rigidity to enable cutting of thin sections.

Plastic resins — **Paraffin wax** — **Gelatine, Agarose**

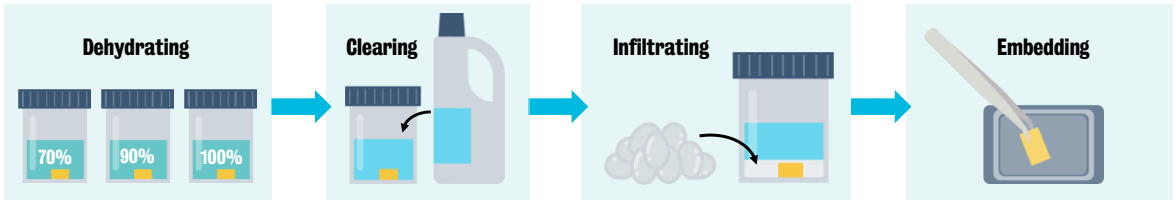
Hard media
Thin section

Soft media
Thick sections



Using paraffin wax

Processing fixed tissue to paraffin wax



Alcohol

Remove alcohol in a solvent that is miscible with alcohol and paraffin wax (xylenes, Histo-clear).

Infiltrate the tissues with molten paraffin wax (replacing the xylene).

Tissues are orientated in metal moulds containing fresh molten paraffin wax and then allowed to cool.

Upon cooling to 4°C, the wax blocks are easily removed from the metal mould, ready for microtomy/storage.

End of part 1