

Module:
Techniques in Neuroscience

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

**Carl Hobbs**

Voice over by Dr Brenda Williams

Topic 1:
**An introduction to
immunohistochemistry**
Part 3 of 4

Part 3

Introduction to part 3

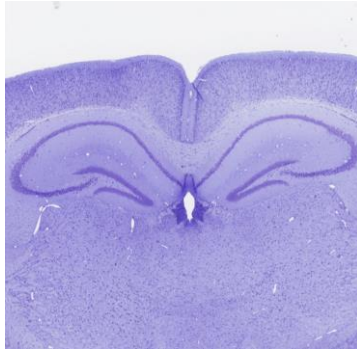


Microscopic examination of tissues requires a cutting of thin sections from the tissue.

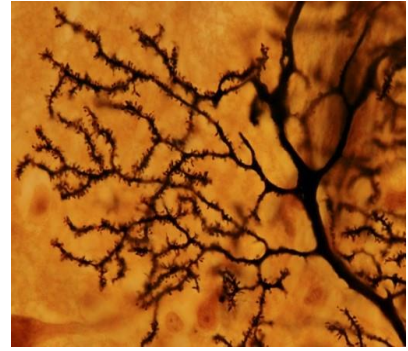


Techniques have been devised that use dyes, heavy metals and fluorochromes to induce colour, or contrast.

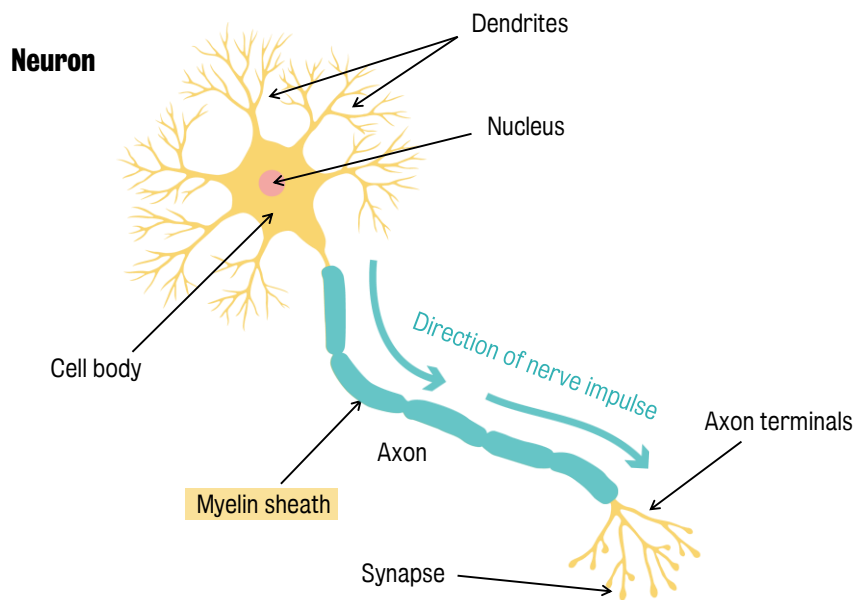
Dye staining



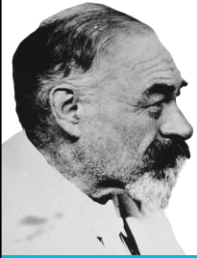
Metal impregnation techniques



Investigating neuron pathobiology



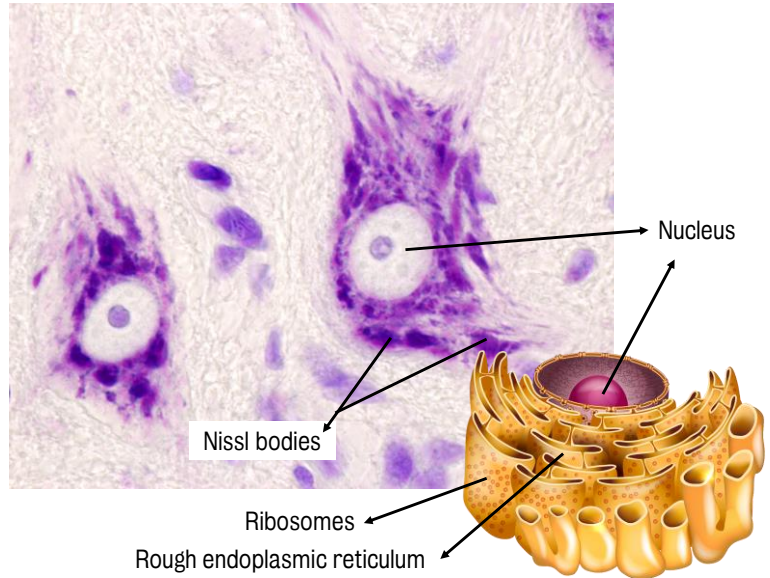
Nissl staining



Staining method developed by the German pathologist Franz Nissl at the end of 19th century

Stains: DNA and RNA in purple or dark blue

Mechanism: ionic interaction



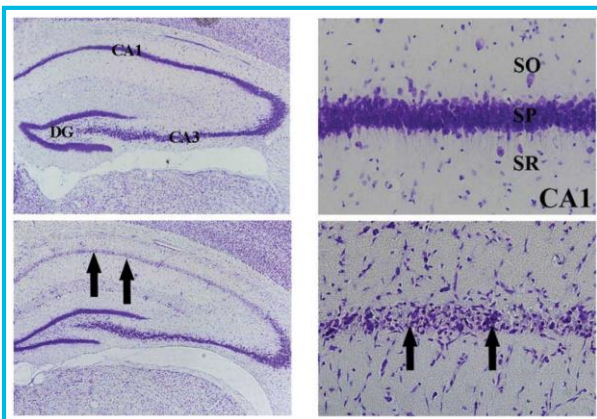
Week 3 Immunohistochemistry: Preserving and studying cells of the brain

Topic 1: An introduction to immunohistochemistry

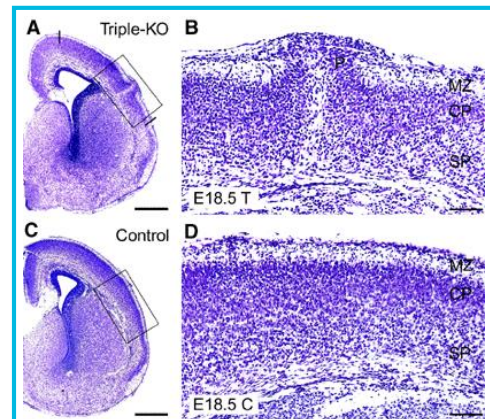
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Nissl staining: applications

Neuronal loss



Abnormal growth and development



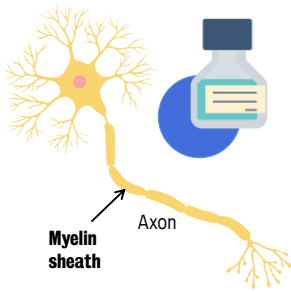
Cho et al. (2015); Herms et al. (2004)

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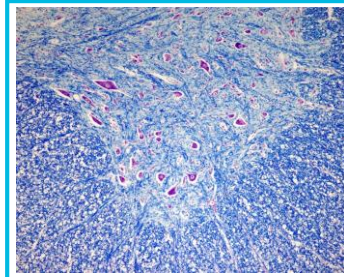
Luxol fast blue



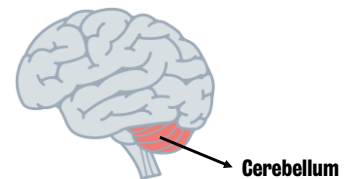
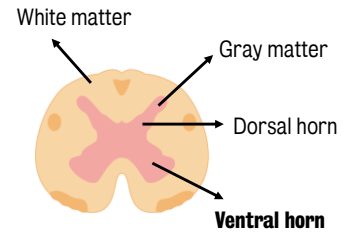
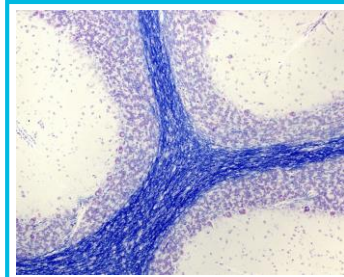
Stains: myelin sheath in blue

Mechanism: ionic interaction between dye (anion) and myelin lipoproteins (cation) initiates binding

Ventral horn of the spinal cord



White matter tracts in the cerebellum



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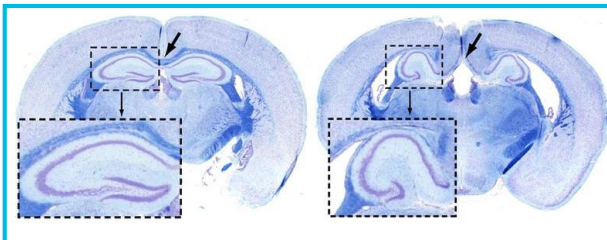
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Luxol fast blue: applications

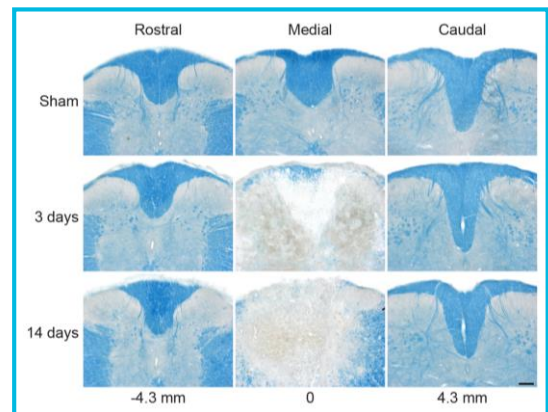


Useful for: the study of the myelinated nerve tracts in the central nervous system and the changes that may occur to myelination

Structure and morphology



Demyelination



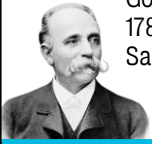
Lacroix et al. (2014); Lavado et al. (2013)

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Metal impregnation: Golgi stain

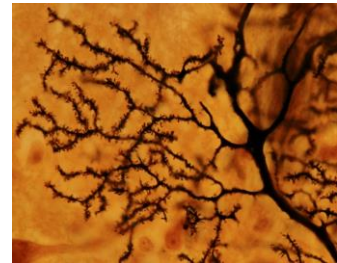
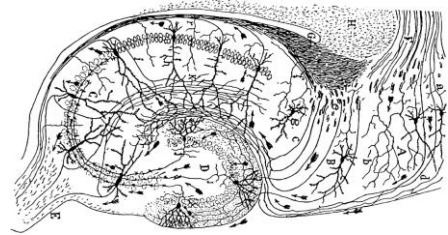
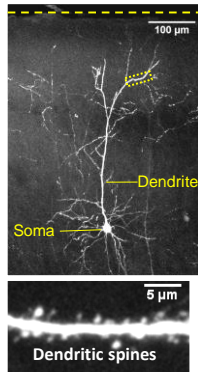


Developed by Camillo Golgi, first published in 1783 and then modified by Santiago Ramon y Cajal



Stains: one to 10 per cent of neurons in dark brown or black

Mechanism: silver precipitate produces insoluble black reaction product



Lacroix et al. (2014); Lavado et al. (2013)

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Golgi stain: applications

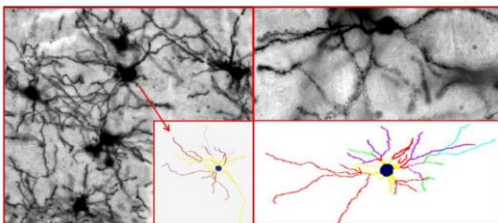


Useful for:
the study of neuronal morphology

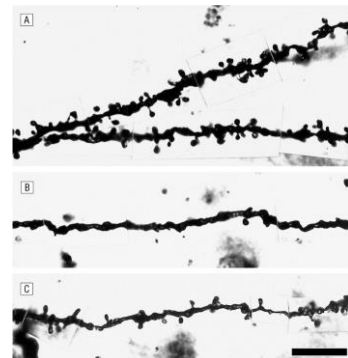


This can be used on very thick sections of the brain.

Neuronal morphology



Dendritic spine morphology and quantification



Giantz & Lewis (2000); Milatovic et al. (2010)

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List of other staining techniques

Myelin**Oil red O:**

- frozen sections only. The majority of fats are destroyed by Paraffin wax processing the dye, when dissolved in 70 per cent alcohol has a preferential solubility for the fat

Solochrome cyanine:

- much simpler to use than LFB method giving a similar myelin positivity

Osmium tetroxide:

- fixed tissues are immersed in 2 per cent Osmium for 2hrs and then processed to P. wax (or snap-frozen and cut as frozen sections)
- oxidation of lipid unsaturated double bonds causes reduction of OsO₄ to metallic osmium
- excellent for studying peripheral myelinated nerve fibres

Marchi's method for degenerating myelin

- Osmium turns degenerating myelin black. Potassium chlorate prevents normal myelin (mostly) from reacting with Osmium and will be unstained. More complicated than dye stains

Click **Next** to continue

List of other staining techniques

Neurons

Several methods utilising Silver nitrate which precipitates as metallic silver on axons: Holmes, Bielschowsky –type methods.

**Glia**

Cajal's gold-sublimate method for astrocytes, Holzer's and Mallory's PTAH methods for gliosis (astrocyte fibrosis/scarring)

Many of the above have been superseded by immunomethods utilising antibodies that have been raised against epitopes specific for a comprehensive range of neural proteins including Neurofilaments (neurons), Glial fibrillary acidic protein (astrocytes, ependymal cells), Iba1 (microglia), Myelin basic protein (myelin) and OLIG2 (oligodendrocytes).

Click **Next** to continue

End of part 3