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## Histology / Immunohistochemistry Targets

**Infarct volume** (TTC, H&E, or Cresyl Violet stain)

**IBA-1** (labels activated microglia)

**TUNEL** (DNA fragmentation - apoptosis)

**Caspase 3** (part of apoptotic activation process – apoptosis)

**GFAP** (Glial fibrillary acidic protein - labels astrocytes)

**NeuN** (labels neurons)

**Doublecortin** (labels new, migrating neurons)

**BDNF** (Brain derived neurotrophic factor - nerve growth factor)

**Luxol fast blue** (stains myelin)

**Myelin Basic Protein** (myelin)

**Synaptophysin** (synaptic vesicle protein – target: synapses)

**Ki-67** (nuclear protein – labels proliferating cells)

**PSA-NCAM** (neural cell adhesion molecule – neuronal adhesions, neuronal growth)

**Olig2+** (oligodendrocyte lineage transcription factor – labels immature neural cell precursor)

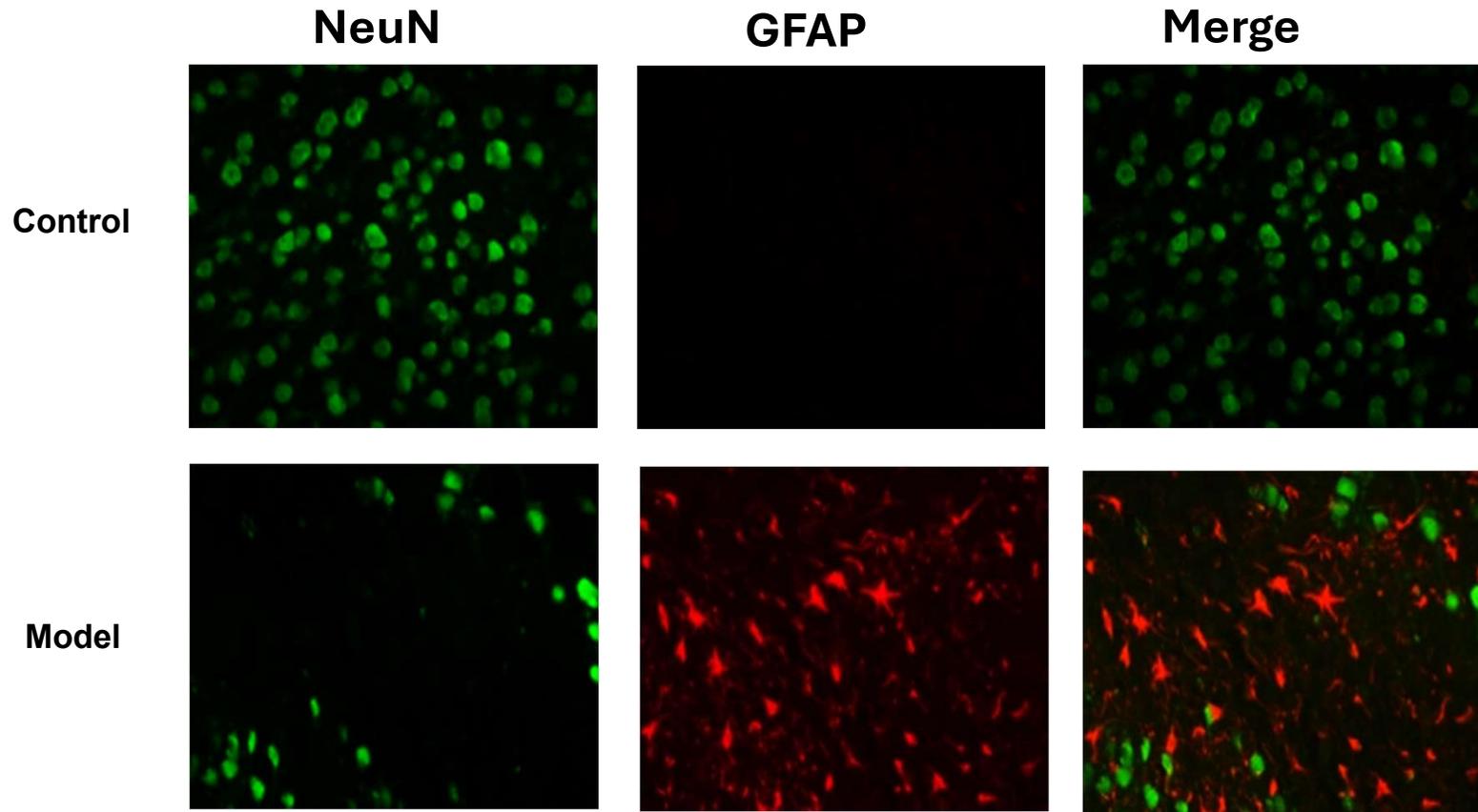
**SOX10+** (transcriptional regulator for oligodendrocyte differentiation – immature oligodendrocytes)

**GST $\pi$**  (glutathione-S-transferase; labels immature oligodendrocytes)

**Fluoro-Jade B** (degenerating neurons)

**3-nitrotyrosine** (inflammation/neurodegeneration/oxidative stress)

## Neonatal HI Model – Cortex (40x)

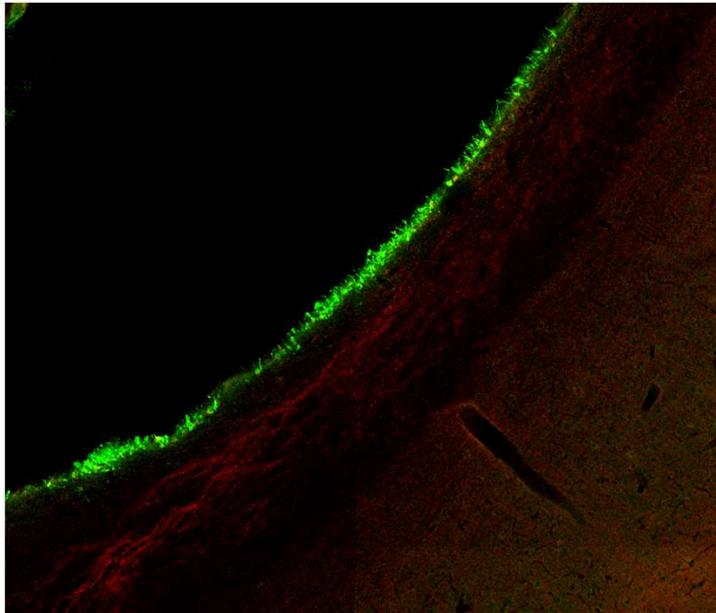


Double Labelling: NeuN (neurons; green) & GFAP (glial cells; red)

# Fluorescent IHC: New Neuron Markers Synaptic Markers

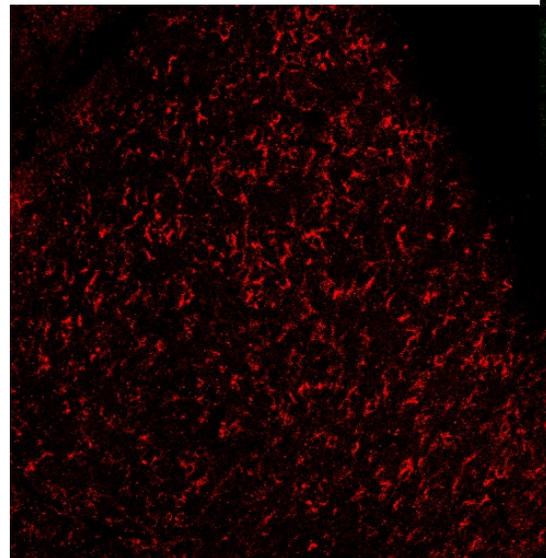
## Neonatal HI Model

### Subventricular Zone

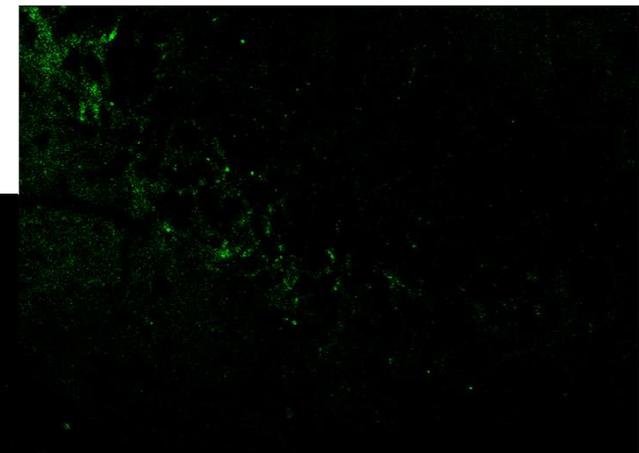


Double Labelling: Ki-67 (proliferating cells; green) & Doublecortin (new migrating neurons; red)

### Hippocampus



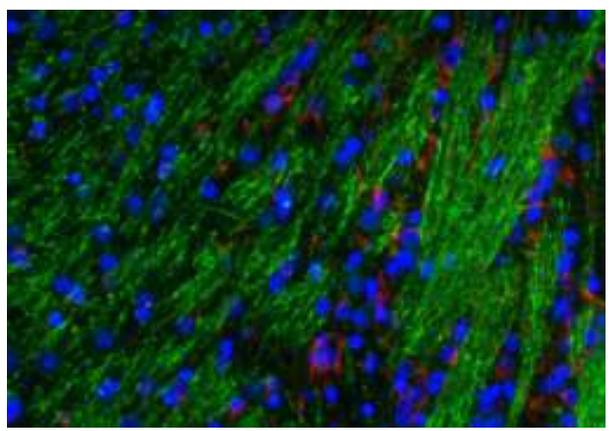
Synaptophysin (synaptic terminal marker; synaptogenesis)



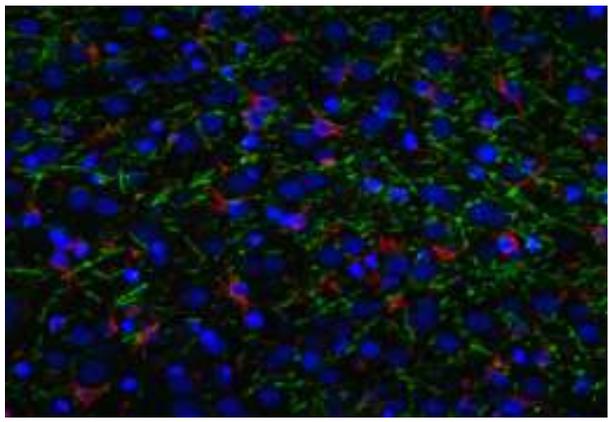
PSA-NCAM (developing/migrating neurons & synaptogenesis)

# Fluorescent IHC: Myelin & Oligodendrocytes

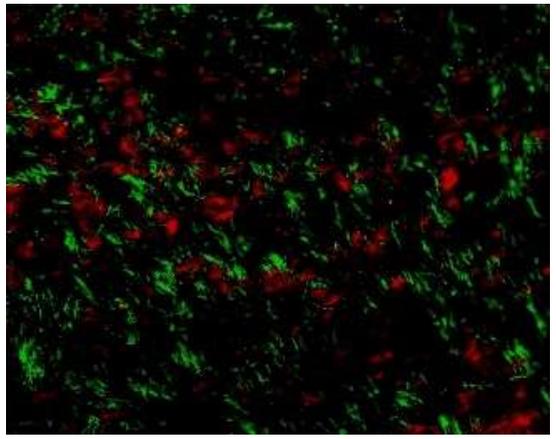
**Control**



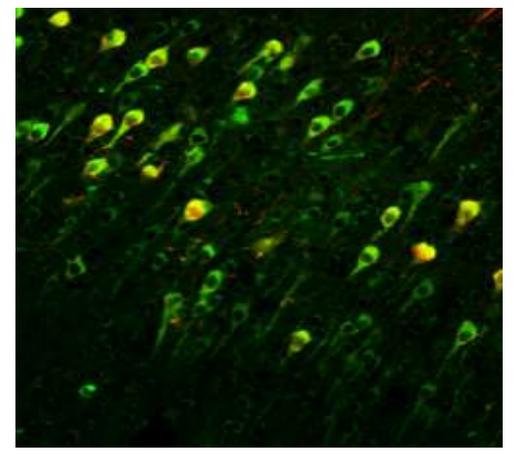
**Model**



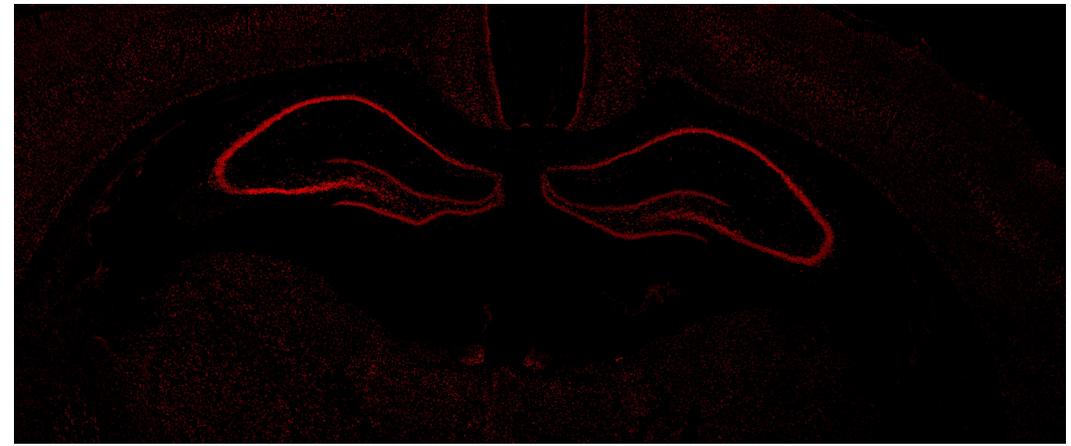
**Green** = Myelin Basic Protein (myelin)  
**Red** = GFAP (glia) **Blue** = DAPI (nuclei)



**Green** = Myelin Basic Protein (myelin)  
**Red** = SOX10 (oligodendrocyte transcription factor)



**Red** = GST $\pi$  (immature oligodendrocytes)  
**Green** = GFAP (glial cells/astrocytes)



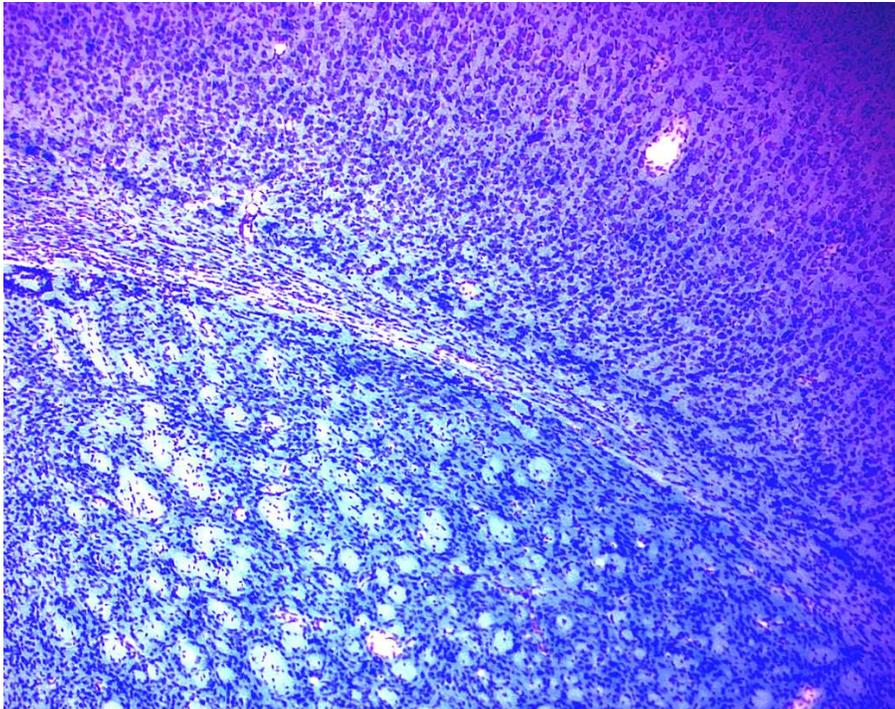
Olig2+ (Oligodendrocyte lineage transcription factor 2 ; immature cell precursor)

## Myelin Stains:

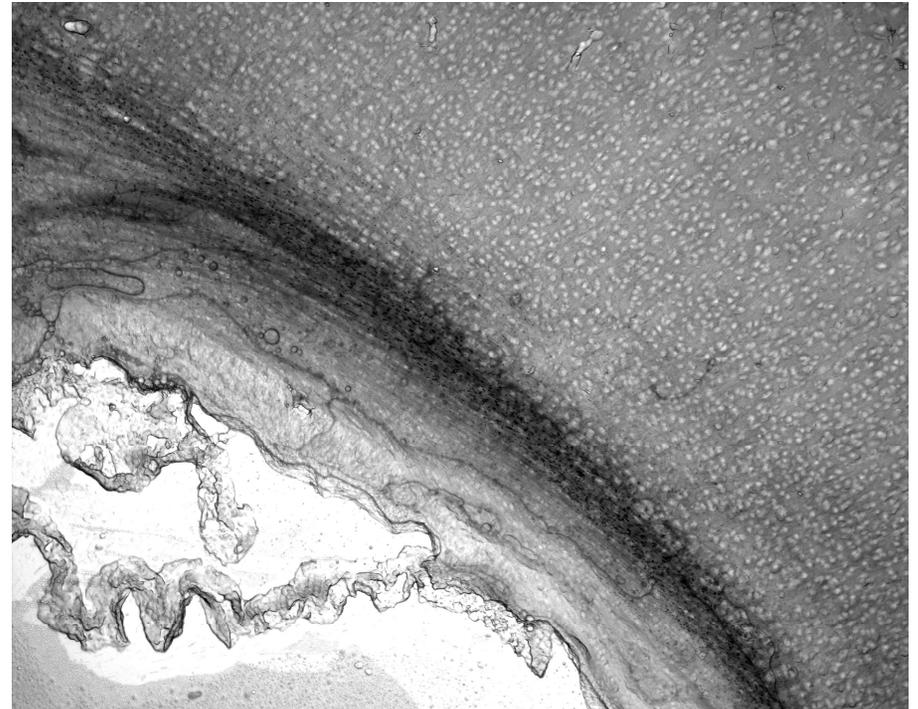
### Luxol Fast Blue & Sudan Black B

## Neonatal Rat Myelination

Myelination is one of the last events in the maturation of the central nervous system; in rat it begins in gestation, and continues throughout the postnatal period, reaching adult levels at approximately 35 days of age, with different developmental rates in different regions of white matter.



Luxol Fast Blue / Cresyl counterstain. Luxol Fast Blue is attracted to hydrophobic domains of protein molecules in myelin (i.e., lipoproteins)

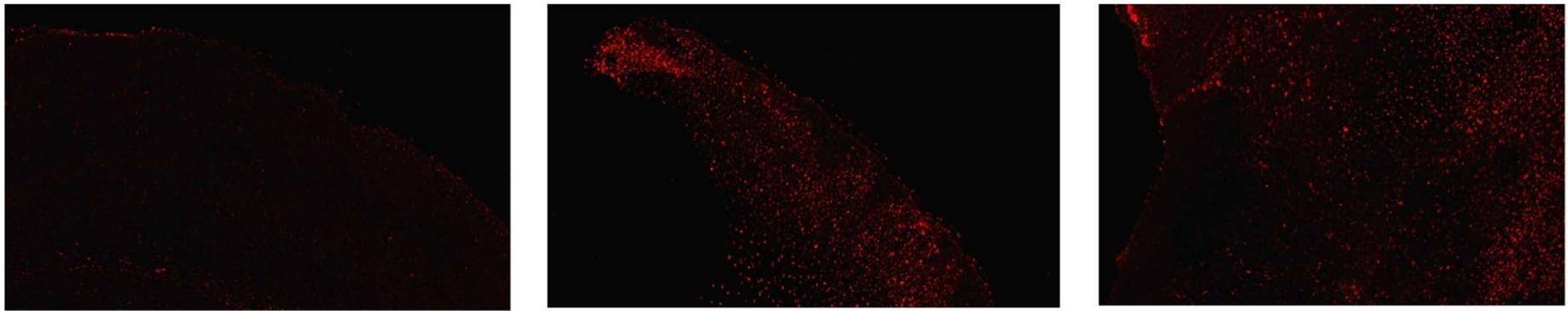


Sudan Black B is used to stain phospholipids and intracellular lipids. It will stain most types of lipids including the phospho- and sphingolipids of normal myelin.

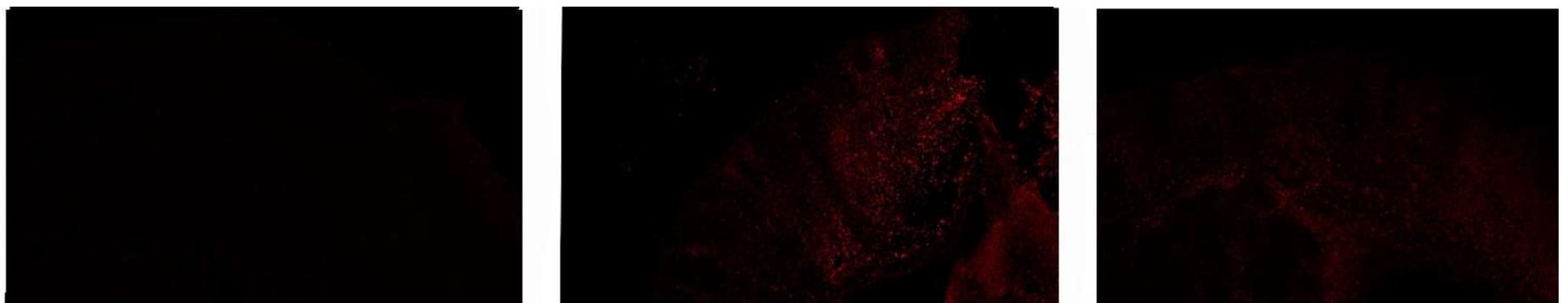
Mature, dry myelin is comprised of approximately 30% protein (e.g. myelin basic protein, oligodendrocyte-specific protein, proteolipid protein) and 70% lipids. Hydrophilic phospholipids and glycolipids account for about 70% of the lipid content of myelin; the remainder is mostly cholesterol, which is more hydrophobic.

# Fluorescent IHC: Iba-1 in Brain

A.



B.



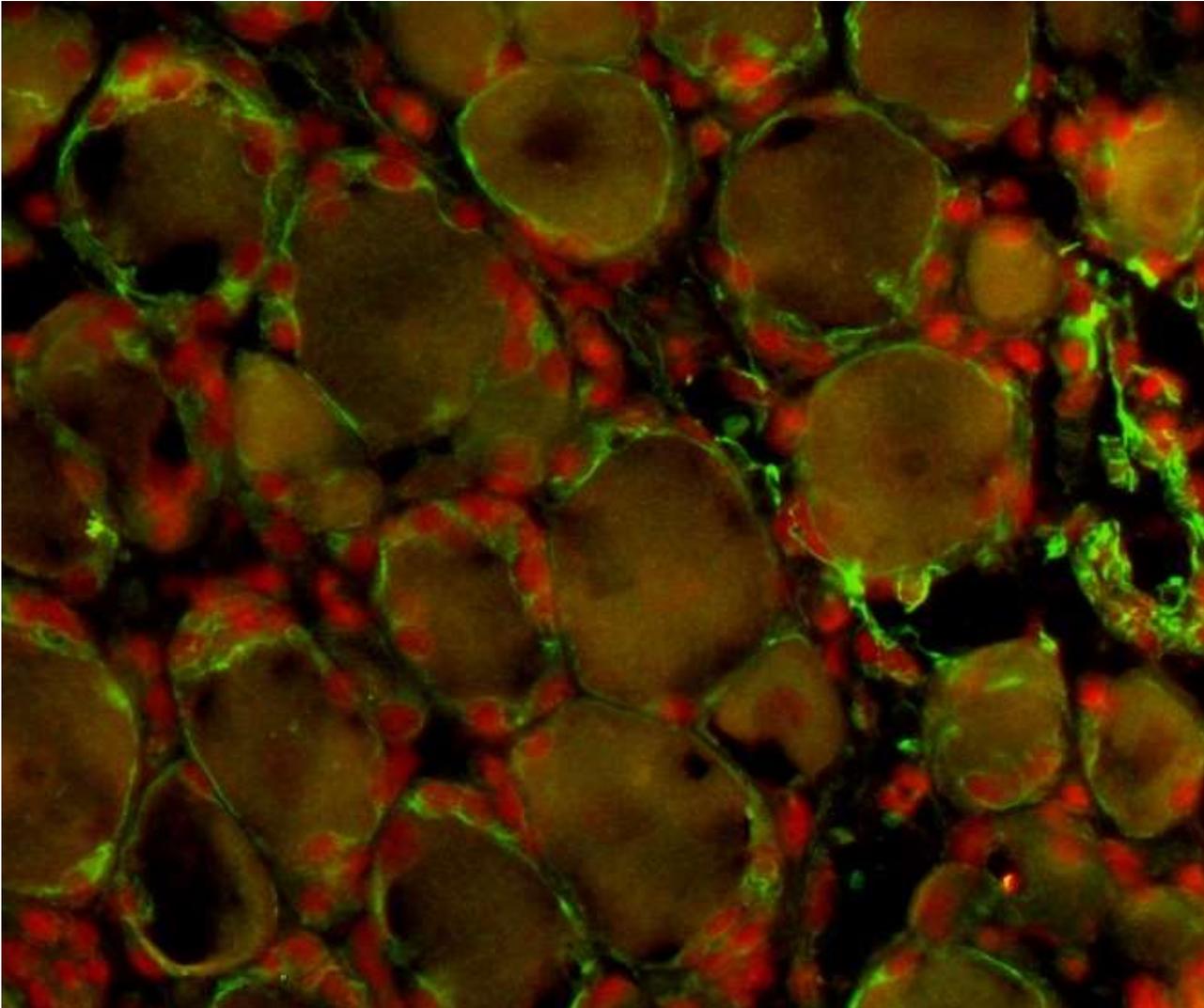
SHAM

HI Control

Test Article

Iba-1 expression in the peri-infarct region of the ipsilesional hemisphere for SHAM , Stroke Control, and treated rat brain at (A) 3 days and (B) 7 days post stroke.

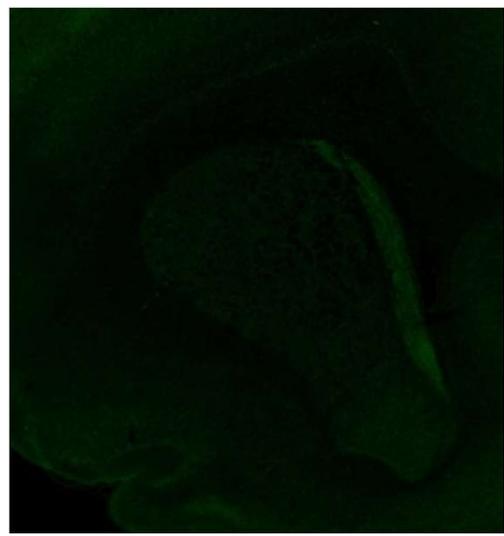
## Fluorescent IHC: Iba-1 in DRG



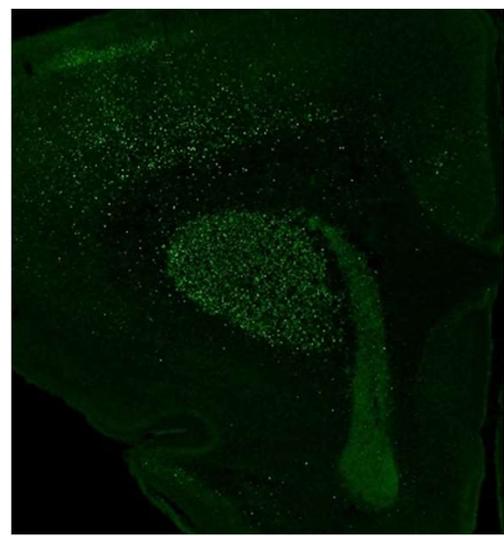
Iba-1 (red) and GFAP (green) expression in rat dorsal root ganglion.

# Fluorescent IHC: Cell Death & Neurodegeneration

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)



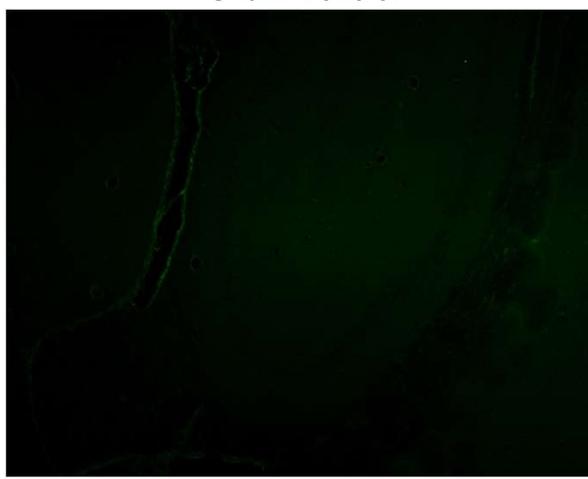
Sham Control



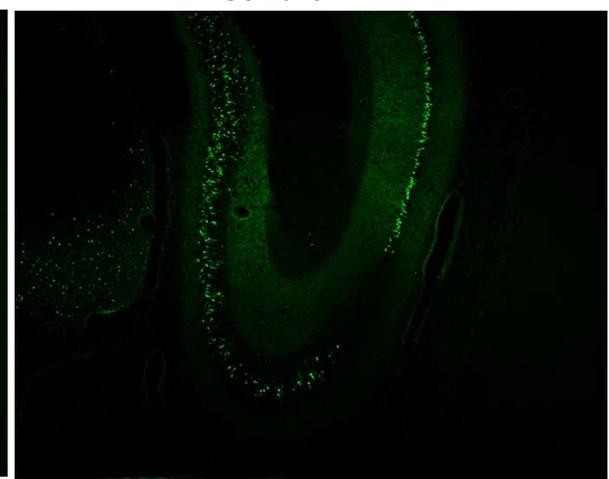
Stroke

Fluro-Jade B

Sham Control



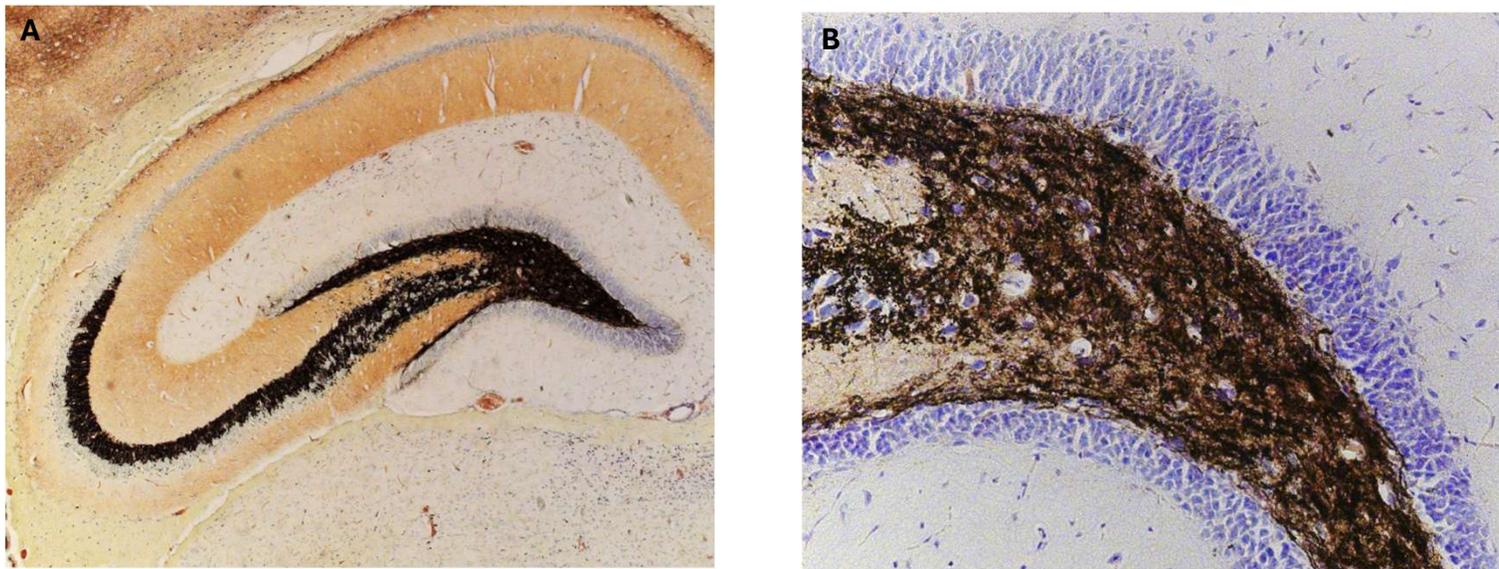
Seizure



FluroJadeB Staining: Neurodegenerative activity in the hippocampus at 4 days post-seizure

## Specialty Staining: Timm Stain

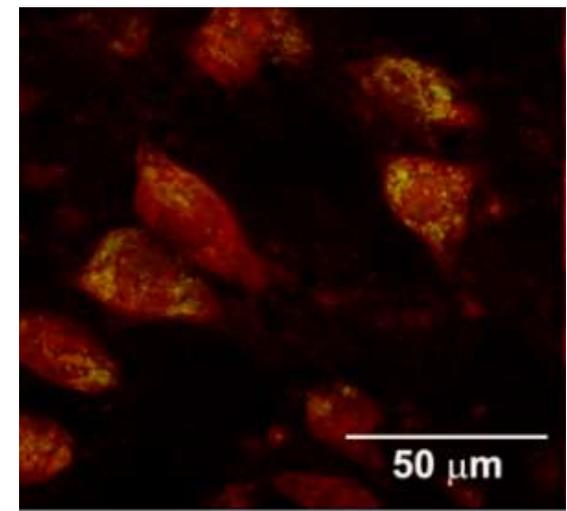
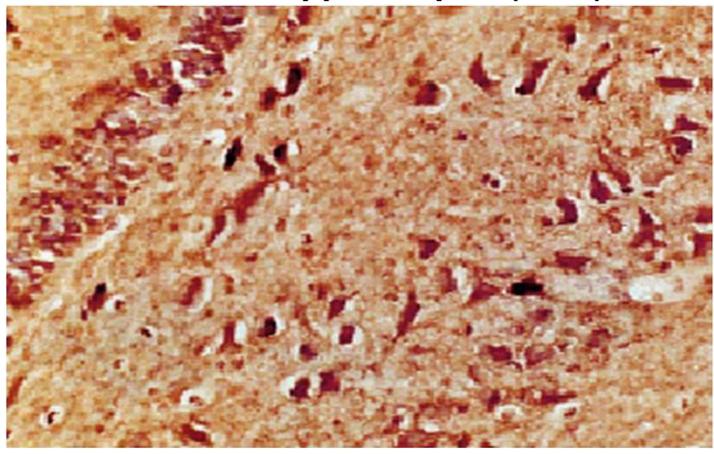
Timm staining may be used to visualize a variety of metals in brain, and is particularly useful for labelling zinc-containing neurons and detecting newly sprouted axons and axon terminals in hippocampus of rodents that have experienced seizure activity.



**Figure 1:** Timm stain counterstained with cresyl. (A) 5x dorsal hippocampus. (B) 20x dorsal hippocampus.

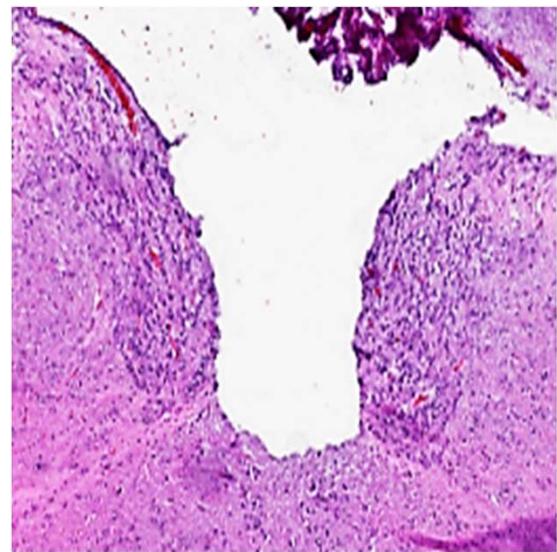
## Other Offerings:

**Somatostatin – Hippocampus (DAB)**

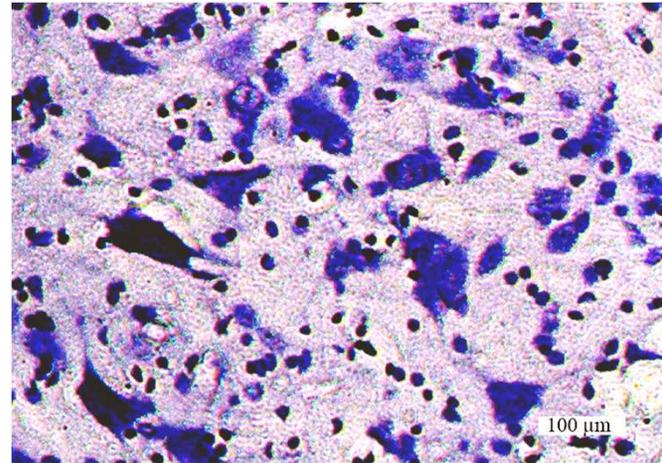


Insoluble synuclein aggregates localized within TH-positive neurons. red = TH; green = proteinase K-resistant  $\alpha$ -synuclein

**H&E Stain**

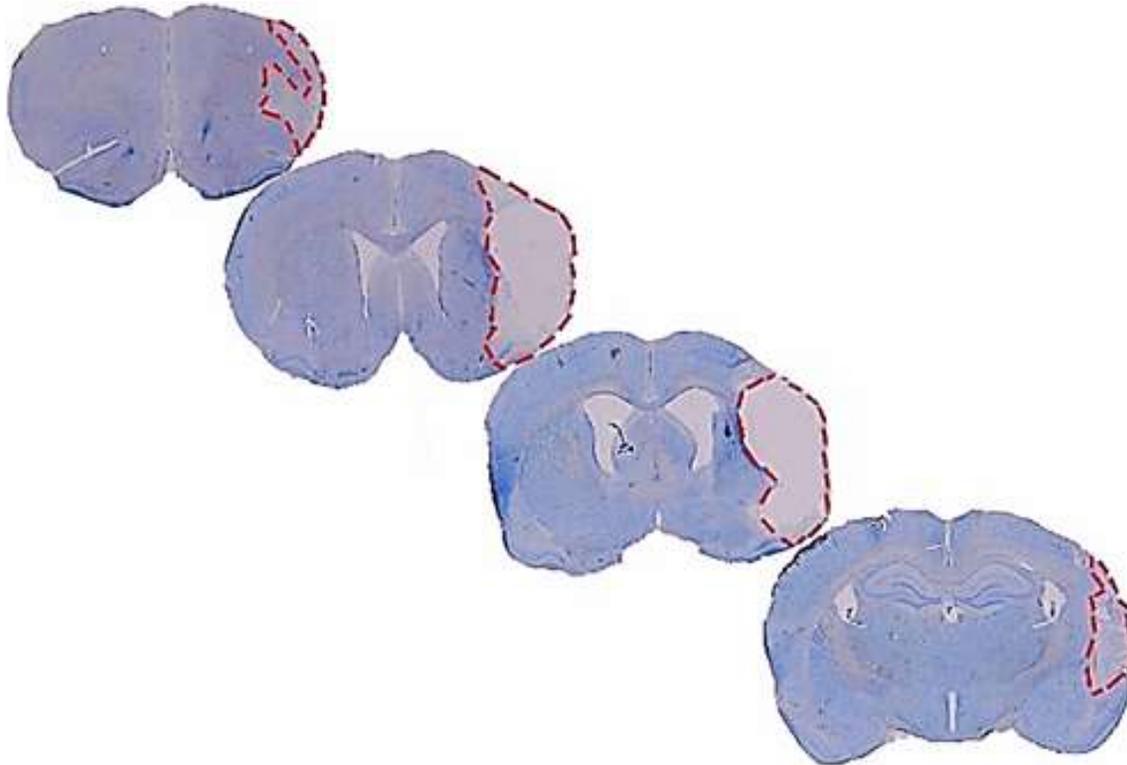


**NISSL Stain – Ventral Horn Spinal Cord**



## **MCA Occlusion - Volumetrics**

Serial 100  $\mu\text{m}$  thick coronal sections are cut using a cryostat ( $-20^{\circ}\text{C}$ ) with one in every 3rd section stained and evaluated to determine infarct volume ( $\text{mm}^3$ ).



A representative example of infarct size and location post-model induction with brain sections (100  $\mu\text{m}$ ) stained with cresyl violet. Infarcted areas in the ipsilesional hemisphere are delineated with hashed lines.