2015 CALENDAR

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Pig Brain
(*Sus scrofa domesticus*)

Premier Neurohistology Services
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GFP Antibody Staining of a Pyramidal Cell in Rat Cerebral Cortex

The gene for expressing GFP (green fluorescent protein) is often incorporated into a viral vector along with the gene for another protein (a "test article") and introduced into experimental animal brains. GFP plays the role of a "reporter of gene expression". The neuron in this image has incorporated the viral vector and the GFP protein produced filling the neuron in a Golgi-like fashion. Even the fine details of dendritic spines are made visible.
Amino Cupric Silver Counterstained with NeuN Staining
Degenerating neurons are represented by the large black, silver-impregnated profiles; smaller black dots are fragments of synapses, dendrites or axons.

NeuN antibody staining (amber) stains the nucleus very darkly and the cytoplasm more lightly.
May is ALS Awareness Month.
Learn more about this disease at www.alsa.org

Amyotrophic lateral sclerosis (ALS) is a terminal motor neuron disease first described in 1869 by Jean-Martin Charcot. This disease gained notoriety when American baseball player Lou Gehrig announced his diagnosis and retirement from the sport in 1939. ALS occurs throughout the world affecting individuals of different races, ethnicities, and socioeconomic conditions. Additionally, there are some commonalities with Alzheimer’s Disease and Parkinson’s Disease, including highly sensitive neurons and their response to stress factors; reduced protein recycling; neuroinflammation; and the transmission of toxic proteins between neurons.
TAU pSERINE 422 MONKEY CORTEX
Pyramidal neurons in monkey cerebral cortex stained with an antibody against Tau protein phosphorylated at serine 422 (Phospho-Tau422).
Gallyas stain for ‘neuropil threads’
The Gallyas stain reveals within neurons neurofibrillary tangles and outside the neurons (in the parenchyma) ‘neuropil threads’ contained within neuronal processes.
Method To Improve Handling Characteristics Of Free-Floating Brain Sections After Immunohistochemical Staining For Amyloid

*Zurhellen C., Tipton, B., Baun, J., Switzer III, R. C.

A PDF copy of the entire poster presented at Society for Neuroscience in 2013 is available on our website: www.nsalabs.com/formaldehyde.pdf

METHODS
1. Removal of sections into rinses (3x5') with Tris-buffered saline* (TBS)
2. 3.7% Formaldehyde-TBS (1:9 dilution of 37% formaldehyde in TBS)
3. Rinses TBS (3x5')
4. Peroxide-TBS- 30'
5. Rinses TBS (3x5')
6. Formic Acid (undiluted from bottle ~95%)- 15'
7. Rinses TBS (3x5')
8. Proceed to serum blocking step (if used) and remainder of procedure

*TBS normally is used in NSA’s antibody protocol instead of PBS

COMPARISON
Below, images compare the untreated vs. the pre-treated near-adjacent sections from a cynomolgous monkey brain embedded in NSA’s MultiBrain® gelatin matrix.

<table>
<thead>
<tr>
<th>No Pretreatment with Formaldehyde</th>
<th>Pretreated with Formaldehyde</th>
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</thead>
<tbody>
<tr>
<td><img src="image1" alt="Untreated Section" /></td>
<td><img src="image2" alt="Pretreated Section" /></td>
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In the left image, wrinkles are the result of differential shrinkage or the expansion between tissue and the gelatin matrix. In the right image, no wrinkles persist in a section treated with formaldehyde prior to formic acid treatment.

Mounted sections stained with eosin reveal relationship of tissue to the gelatin matrix.

Wrinkles shown by circles (left) indicate differential shrinkage/expansion of tissue with respect to the gelatin matrix whereas the treated tissue (right) displays no shrinkage/expansion.

Formaldehyde treatment of free-floating tissue sections, that are pretreated with formic acid in preparation for staining with Aβ-related antibodies, results in sections that are easier and faster to mount and yield better quality.

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**Antibody Staining for Tau Oligomers in Alzheimer’s Disease**

The antibody T22 reveals oligomers of Tau (aggregates/clusters of a ‘few’ Tau proteins) which are currently thought to be the most disruptive of the aberrant forms of Tau.

Section from the cortex of a human Alzheimer's patient.
Yellow Fluorescent Protein (YFP) Antibody Staining of Pyramidal Neurons in Rat Basolateral Amygdaloid Nucleus

YFP is a mutant variant of Green Fluorescent Protein (GFP). The gene for expressing these proteins is often incorporated into a viral vector along with the gene for another protein (a "test article") and introduced into experimental animal brains. YFP or GFP play the role of a "reporter of gene expression".
The c-Fos protein is rapidly produced following a variety of external stimuli and its gene is therefore termed an 'immediate early gene'. Staining for c-Fos soon after the administration of a candidate neuro-active drug is used to reveal the neurons that respond to the drug. This image shows a widespread neuronal response.
Microglia in Stroke Damage (Rat Brain)

The antibody, Iba1 (green in this image), reveals microglia whether in a surveillance (‘resting’) state or in a state of hypertrophy indicating a reactive state. The antibody, CD68 (red in this image), is often considered a marker for ‘reactive’ microglia. This image reveals that not all hypertrophied (reactive) microglia stain positive with the CD68 antibody.