Brain Microanatomy: Artifacts, Neoplastic Lesions and Utilization of Special Stains

Current Standard for CNS Evaluation in Rodent Toxicity Studies
ESTP Congress in Ghent, Belgium
12 September 2013

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Neurocytology, Including Cell Types, Cell Reactions and Artifact

**Cells of Neuroectodermal Origin**
- Neurons
- Astrocytes
- Oligodendrocytes
- Ependymocytes

**Cells of Mesenchymal Origin**
- Microglia (as well as blood vessels, adipose tissue, and meninges)
Large Motor Neurons
Large Sensory Neurons
Mixtures of Large Neurons and Small Neurons (Granule Cells)
Olfactory Bulb

Cochlear Nucleus
Locations of all 7 circumventricular organs in a rat brain (LFB/CV stain)
6 CVOs from a rat brain

a = organum vasculosum of lamina terminalis

b = subfornical organ

c = median eminence

d = subcommissural organ

e = pineal

F = area postrema

Necrotic neurons versus Artifactual Dark Neurons

“Red Dead” (Eosinophilic) Neuron (left)

Dark Neuron - representing a handling artifact - (right)
Dark neurons tend to cluster
Dark Neurons Are most often the result of handling.
Drying Artifact

Saline Artifact
Dark neuron artifact is most apparent in large cytoplasm-rich neurons.
In cresyl violet-stained sections, dark neurons are not degenerative!
Eosinophilic Neurons

(H&E)

Eosinophilic Neurons

(Cresyl Violet)
Dark Neurons vs. Dead Neurons

1) Dark neurons are monomorphomic; eosinophilic neurons are characterized by different stages of degeneration. (The only exception may shortly after a peracute injury.)

2) Eosinophilic neurons are accompanied by additional changes such as secondary cellular reactions and, occasionally, an altered neuropil.
Unilateral ischemic insult, rat brain
Eosinophilic neurons in different stages of degeneration
Swollen neuron, eosinophilic neurons, karyorrhexis and active microglial cell
Fluoro-Jade Stains for Degenerating Neurons

1) Lower the threshold (magnification) for detection.
2) Allow observation of degenerative neuronal processes as well as cell bodies.
3) Allow for the detection of small-sized degenerating neurons for which it is difficult to detect an eosinophilic cytoplasmic change.
4) Assist in the detection of peracute degenerative processes (prior to development of eosinophilia).
Retrosplenial cortex (rat) 48 hrs. after MK-801 treatment
Retrosplenial cortex (rat) 48 hrs. after MK-801 treatment

(H&E- vs. Fluoro-Jade B-stained sections)
Hippocampal CA1 sector (rat) 24 hrs. after fentanyl treatment

(H&E- vs. Fluoro-Jade B-stained sections)
CA1 sector of a rat hippocampus 48 hours after a “drug treatment”.

H&E vs. Fluoro-Jade B for detecting degeneration in small-sized interneurons.
Higher magnification images for degenerating small-sized interneurons.
Peracute injury (due to a one-hour grand mal seizure).
Adenosine A1 KO mouse killed after a one-hour grand mal seizure.

H&E vs. Fluoro-Jade C
Dark Neurons in H&E and Fluoro-Jade-stained sections

H&E

Fluoro-Jade
1. Frozen sections are necessary for silver degeneration stains. F-J stains may be used on frozen or paraffin sections.

2. Silver degeneration stains require greater technical skill/may have more artifactual staining.

3. Silver degeneration stains are archivable. (Fluoro-Jade will fade while viewed with fluorescent light, although the shelf life of stained slides is fairly long.)

4. Silver degeneration stains show greater neuronal process detail.

Section from the same rat and the same (contralateral) brain level stained with amino cupric silver.
“Unilateral Optic Nerve Atrophy” in a rat (H&E, F-J B and cupric silver)

Different levels of midbrain (and different time points) showing axonal injury with retraction ball formation.
For Axonal Injury

- Silver degeneration stains (e.g. amino cupric silver) are the stains of choice if you can use frozen sections.
- Amyloid precursor protein (APP) stains are O.K. for peracute axonal injury if you are limited to doing IH on paraffin sections.
- Non-degenerative silver stains such as Bodian’s, Bielschowsky’s, Gallyas’ are also good for axonal injury.
Non-degenerative silver stain for internal neuronal structure (Bielschowsky’s)
Astrocytes

1. Provide nutritional support of neurons and maintenance of extracellular electrolyte & neurotransmitter levels. Crucial for neuron function and survival.

2. Signal other astrocytes via gap junctions.

3. Have neurotransmitter receptors similar to those of neurons.

4. Are an important component of the BBB & also regulate blood flow.

5. Principle marker = GFAP.
Normal-appearing astrocytes, H&E-stained section of cerebral cortex.
Examples of reactive and gemistocytic cytoplasm-rich astrocytes.
Normal GFAP-stained astrocytes ("star cells"), cerebral cortex.
Reactive astrocytes have thickened cytoplasmic processes.
"Hippocampal sclerosis", CA1 sector (GFAP)
Multiple brain sections from a clinically normal rat!!!
Vacuoles: Artifact or a Peracute Alteration?
Cerebellar Vacuoles; H&E, GFAP and Fluoro-Jade B.
One Example of “Good Artifact”

Dogg cerebral cortex, H&E stain.

Alzheimer type II astrocyte change due to experimentally-induced hyperammonemia.

Images courtesy of Dr. Michael D. Norrenberg
Are these changes also examples of “good artifact”?
Two Types of Brain Edema

Francesca & Rezzani (2010). Aquaporin and blood brain barrier

*Curr Neuropharm* 8:92-96
GFAP (Green) vs. Aquaporin 4 (Red)

Astrocytes and Aquaporins in the Pathogenesis of Cytotoxic Brain Edema and/or in Postmortem Swelling of Astrocytes

1) Astrocyte nuclear or cytoplasmic swelling may occur in certain metabolic diseases.

2) Astrocyte swelling (especially cytoplasmic) also represents a very common postmortem artifact.

3) This swelling may be enhanced by certain pharmacologic agents.
Oligodendrocytes

1. Myelin-forming cells of the CNS.

2. One oligodendrocyte myelinates multiple axons (as many as 50 or more).

3. Para-neuronal oligodendrocytes are often referred to as “satellite cells.”
Left: Classic Artifactual “Fried Egg” Appearance of Oligodendrocytes

Right: Normal Oligodendrocytes in a Perfusion-fixed Brain
Left side micrograph: Within the brain’s fiber tracts, oligodendrocytes are typically arranged in rows.

Right side micrograph: Some cranial nerves show an extra-axial transition between a central (right) to a peripheral (left) pattern of myelination.
Examples of Neoplastic Satellitosis
Microglia

1. Are the inflammatory cells of the CNS comparable to the blood monocytes and tissue histiocytes.
2. Constitute 5 – 20% of the brain cells.
3. Function in both pro-inflammatory (“classical activation” and reparative (“alternative activation”) modalities.
4. Ionized calcium binding adaptor molecule 1 (Iba1) is often used to stain reactive microglia.
Find the single microglial cell in this section of normal cortex. H&E
Neuronophagia by activated microglial cells
Progression of neuronophagia by microglial cells
Microglia reacting to necrotic neurons – CA1 sector of hippocampus
Reactive microglial cell, cerebral cortex

Iba1 Stain, cerebral cortex
Ventricular fibrillation/cardiac arrest model, rat (14-days prior to death)

H&E

Iba1
Principal Histologic Artifacts in Brain Sections

1) **Dark Neurons**

2) **Vacuolation of neurons, neuropil or myelin**
Good (left) versus suboptimal preservation of DRG by perfusion fixation
Prominent vacuoles found sporadically in sensory ganglia of rats.
Buscaino bodies ("Mucocytes")

An artifact of myelin.

H&E
Contrasting Two Patterns of Intramyelinic edema
Buscaino bodies are occasionally refractile under polarized light.
Brain Microanatomy: Neoplastic Glial Lesions in Rodents

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Classification of Proliferative Lesions

• Neuronal
  – Medulloblastoma
  – Neuromyoblastoma

• Glial/Schwann Cell
  – Astrocytoma
  – Glioma
  – Oligodendroglioma
  – Schwannoma

• Choroid plexus
  – Papilloma
  – Carcinoma

• Ependyma
  – Ependymoma

• Meninges
  – Granular cell aggregates
  – Granular cell tumour
  – Meningioangiomatosis
  – Meningioma

• Other cell lineages
  – Lipomatous hamartoma
  – Malignant reticulosis
  – Lymphoma
### Historical Control Data* for Brain from the NTP

<table>
<thead>
<tr>
<th>F344 RATS – Brain Tumor</th>
<th>Incidence (M)</th>
<th>Incidence (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASTROCYTOMA, High grade (aka. Malignant)</td>
<td>3/1248</td>
<td>0/1200</td>
</tr>
<tr>
<td>EPENDYMOMA, benign</td>
<td>1/1248</td>
<td>0/1200</td>
</tr>
<tr>
<td>EPENDYMOMA, malignant</td>
<td>0/1248</td>
<td>1/1200</td>
</tr>
<tr>
<td>GLIOMA**, low grade (aka. Benign)</td>
<td>0/1248</td>
<td>1/1200</td>
</tr>
<tr>
<td>GLIOMA**, high grade (aka. Malignant)</td>
<td>0/1248</td>
<td>2/1200</td>
</tr>
<tr>
<td>OLIGODENDROGLIOMA, low grade (aka. Benign)</td>
<td>1/1248</td>
<td>0/1200</td>
</tr>
<tr>
<td>OLIGODENDROGLIOMA, high grade (aka. Malignant)</td>
<td>1/1248</td>
<td>3/1200</td>
</tr>
<tr>
<td>GRANULAR CELL TUMOUR, BENIGN</td>
<td>2/1248</td>
<td>0/1200</td>
</tr>
<tr>
<td>SCHWANNOMA, MALIGNANT</td>
<td>1/1248</td>
<td>0/1200</td>
</tr>
</tbody>
</table>

- ** Has been used for ‘glioma, mixed’ or ‘astrocytoma’
### Historical Control Data* for Brain from the NTP

<table>
<thead>
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<th>B6C3F1 MICE – Brain Tumour</th>
<th>Incidence (M)</th>
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<tbody>
<tr>
<td>MENINGIOMA, benign</td>
<td>1/1150</td>
<td>0/1197</td>
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<td>1/1150</td>
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Glial Cell Neoplasms in Rodents

1) “Astrocytomas” are the most common glial tumor in rats, followed by oligodendroglioma and mixed glioma

2) Primarily seen in older-aged rats (15 months +)

3) Immunohistochemistry suggests a histiocytic cell lineage for astrocytomas in rats, so the new terms that have been recommended are “malignant microgliial tumor” or “microglioma”.

Astrocytoma: malignant, high grade

Photo courtesy of Dr. James Morrison.
Astrocytoma: H&E, protoplasmic morphology

Photo courtesy of Dr. James Morrison.
Astrocytoma: fibrillary morphology

Photo courtesy of Dr. James Morrison.
Astrocytoma: neuronal satellitosis

Photo courtesy of Dr. James Morrison.
Astrocytoma: extension into meninges

Photo courtesy of Dr. James Morrison.
Astrocytoma: necrosis and palisading

Photo courtesy of Dr. James Morrison.
Astrocytoma: cellular atypia

Photo courtesy of Dr. James Morrison.
Oligodendroglioma:

Photo courtesy of the NTP
Oligodendroglioma:

Photo courtesy of the NTP
Oligodendroglioma:

Photo courtesy of the NTP
Oligodendroglioma:

Photo courtesy of the NTP
Oligodendroglioma:

Photo courtesy of the NTP
Mixed Glioma:

Photos courtesy of the NTP
Mixed Glioma:

Photo courtesy of the NTP
Mixed Glioma:

Photo courtesy of the NTP
Immunohistochemical Staining of Rat Glial Cell Neoplasms

1) Astrocytoma

2) Oligodendroglioma

3) Mixed gliomas (oligo + astrocytoma)
IHC on Rat Astrocytomas

GFAP IHC  
Photos courtesy of Dr. James Morrison  
Iba-1 IHC
IHC staining of mixed gliomas

Staining for GFAP and for nestin (a marker of astrocyte precursors)

Summary Comments
– Glial Neoplasms in Rats -

• Glial cell neoplasms are very uncommon in rats and are rare in mice; these neoplasms are primarily found in old rats.

• The most frequent glial cell neoplasm in the rat is the “astrocytoma”, but IHC has shown the tumor cells to stain for monocyte markers such as Iba1. The diagnostic terms “microglioma” or “malignant microglial cell tumor” may, therefore, be more appropriate.

• Oligodendrogliomas show IHC markers for oligodendrocytes.

• Some mixed gliomas have been shown to contain both neoplastic oligodendrocytes and GFAP + neoplastic astrocytes or nestin + cells. (Nestin is expressed in proliferating neuroepithelial cells, including astrocyte precursors.)
Neuropathology Overview

1. The brain is a highly heterogeneous organ.
2. Many neuroanatomic regions have unique sensitivities to physical or chemical insult.
3. Adequate evaluation for neurotoxic endpoints requires:
   a) Proper tissue preparation (to avoid artifact)
   b) Relatively extensive sampling
   c) Application of a variety of stains, when appropriate
   d) Pathologist’s time and experience
4. The regulatory agencies need to know which brain regions were examined.
Acquiring new scientific knowledge can be a pleasing experience.

Un-retouched electron micrograph of normal mitochondria within a small myelinated axon.

From: Haines, DE; Fundamental Neuroscience for Basic and Clinical Applications, 3rd Ed. Elsevier, 2006