Evaluation of the Adult Nervous System in Preclinical Studies

Mark T. Butt, DVM, Diplomate, ACVP
President, Tox Path Specialists LLC
20140 Scholar Drive, Hagerstown, MD 21742
301-845-0719
866-902-0771 (Fax)
240-315-7236 (Mobile)
mbutt@toxpath.net
sbutt@toxpath.net

Nervous systems evaluation

• Do the following
  – Look at the right place
  – At the right times
  – In the right ways

The right place

What else other than pathology is going to be done?
Begin with the end in mind: be prepared to harvest whatever samples might be needed to accomplish your goals

Remember:
- Necropsy, like death itself, is irreversible
More often, neurotoxins kill cells in smaller portions of the brain.

MDMA, Alcohol, MPTP, Meth, Kainic acid, ZNH, MPTP, PCP, Domoic acid, PCP.

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Inadequate evaluation of the brain.

For any species, sampling the same number of levels provides comparable representation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Brain length (mm)</th>
<th>Sampling interval (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Using 40 samples</td>
<td>Using 60 samples</td>
</tr>
<tr>
<td>Mouse</td>
<td>12</td>
<td>0.30</td>
</tr>
<tr>
<td>Rat</td>
<td>21</td>
<td>0.35</td>
</tr>
<tr>
<td>Monkey</td>
<td>65</td>
<td>1.08</td>
</tr>
<tr>
<td>Dog</td>
<td>75</td>
<td>1.25</td>
</tr>
</tbody>
</table>

A sampling rate of 50-60 levels per brain offers a balance between a reasonable safety assessment and reasonable effort.

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0.32mm spacing between levels is the interval commonly used in R&D when characterizing effects in a rat brain.

This spacing ensures adequate representation of most populations of the brain.

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Trimming scheme

- The following stains:
  - Hematoxylin and eosin (H&E) stain: all tissues
  - Neurodegeneration stain: brain, spinal cord, dorsal root ganglia, and cervicothoracic and superior mesenteric ganglion (spinal cord and ganglia staining somewhat optional)
  - Microglial stain: IBA-1
  - Glial fibrillary acidic protein (GFAP) stain: brain and spinal cord
  - Luxol fast blue: for myelin
  - Silver stain: brain and spinal cord and possibly nerves

- Peripheral nerves
  - Longitudinal section: paraffin/GMA and H&E
  - Cross section: Spurr’s (or GMA), toluidine blue following osmium post-fixation (even paraffin following osmium post-fixation can be rewarding)
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Trimming scheme - brain

- Oversized, 2x3 inch slides
  - Usually used if there is an implant on one side of the brain
- Regular size slides
  - When there is no difference between the sides of the brain

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Spinal cord

- All prime anatomy of the spinal cord is maintained
- Allows detection of subtle changes in nerve fibers

At the right times...

Multiple time points, including a relatively early time point (2 to 4 days), are necessary to accurately characterize nervous system changes, especially to assess neuronal effects.

Each compound has its own peak opportunity for detectability
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• Rat brain treated with MK801
• Arrows: neurons with very subtle vacuolation in the cytoplasm

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• Rat brain treated with MK801 – 2 days post administration
• Fluoro-Jade B stain allows detection of neuronal necrosis
• When is the right time point?
  – Based on available information
  – Look in a 2-4 day time period

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Imaging in toxicology studies
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The right way…

A battery of special stains will ensure a sensitive evaluation of the nervous system

All tissue changes are not created equal

- 95% of what is seen is either normal, an artifactual change (more about artifacts later), autolysis, or an incidental spontaneous change of no significance

Perfusion: why?

Perfusion fixed

Immersion fixed

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Neuronal necrosis stains: why?

Stains – cupric silver

Stains – fluoro Jade B

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**IBA STAIN**

Reliable way to assess increased microglial activity/microglial activation

**Stains – GFAP**

- Glial fibrillary acidic protein (GFAP) detections of Astrocytes

**Luxol fast blue stain**
- Myelin staining (blue)
- Detection of patchy demyelination areas

**Classic silver stain**
- Staining of filamentous elements

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Special procedures example

Thin sections, firm resin embedding, perfusion fixation, glutaraldehyde and osmium post-fixation

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Summary – priorities (decreasing importance)

• Look at enough brain/spinal cord/peripheral nervous system
• Look early and often;
  Include a 2 -4 days post-exposure time point;
  Use imaging techniques if possible to increase your time points if applicable
• Look with the right stains to detect subtle changes
  – Neuronal degeneration (cupric silver of floros-Jade)
  – Microglial stains
  – GFAP
  – Silver
  – Luxol fast blue
  – Other
• Use perfusion fixation if possible

Thank you’s and acknowledgements

• Thanks for listening
• Thanks to NeuroScience Associates and Northern Biomedical Research for supplying various slides
• Thanks to all my clients for all their great neuro studies over the years

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