Non-neoplastic lesions and Interpretive perspectives.
(Emphasizing examples from the NTP CNS/PNS Non-neoplastic Atlas)

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Some of the most important work we do as industrial veterinary pathologists is protecting the intellectual heritage of our society. Which of these children received the neural developmental opportunity to succeed in life?
Let’s not forget the retiree and lasting mental integrity!
International efforts in diagnostic harmonization has been very active the past year with development of rodent CNS Atlas materials both in Europe and at the NIEHS/NTP.

The CNS neoplastic and non-neoplastic European collection is referenced on-line at goRENI. Global Open Registry Nomenclature Information System and published as:


The NTP rodent non-neoplastic Atlas is close to completion and will be published on-line and available to everyone.

Following are selected NTP archived examples of rodent CNS lesions with interpretive perspectives relevant to NTP studies.
As emphasized by Dr. Rao, knowledge of the normal features & functions of brain regions is an important study prerequisite.

- Hippocampal dysplasia
- Normal hippocampal structure
Sciatic nerve neuronal ectopia
Although neuronal ectopia is seldom recognized as a treatment-related effect in general toxicity studies, the advent of generational studies underscore the importance of recognition of various aberrations of neural development.
Subjective recognition of neuronal loss is difficult and if suspected usually requires morphometric methods in dedicated studies.

A marked example of bilateral reduction of neurons in the Oculomotor nuclei (Cr N 3)
Some Normal
and Incidental Rodent CNS Findings
Normal Islets of Calleja,

They are known to consist of primordial neural cells capable of multiple differentiation pathways to glial and neuronal cells. Granular neurons in these islets have a high density of D3 (dopamine) receptors and may have less understood neurophysiological functions (Ref Owen, G.S. & Halliwell, J.V.).
Normal Circumventricular Subfornical Organ,

This thirst regulation center also targets the Medial preoptic nucleus. Note this organ’s relationship to the fornix above & the III ventricle and the medial habenular nucleus below.

The subfornical organ contains one of the highest concentrations of Atrial Naturetic Factor (ANF/ANP) receptors as well as Angiotensin II receptors in the brain thus combining central baroreceptor and renal control of blood pressure.
Normal Circumventricular Subcommissural Organ,
As part of the roof of the cerebral aqueduct it is commonly misinterpreted as a neoplasm. It functions primarily in release of SCO-spondin, a glycoprotein important in spinal & ventricular development.
Incidental Thalamic Mineralization,

- Note hippocampal dentate gyrus above
- The thalamic distribution is usually bilateral but not necessarily symmetrical.
- It is a more common finding in B6C3F1 mice than in rats.
- Interpretive caution is needed since similar changes may be present with compound related foci of chronic brain necrosis.
- Similar mineral deposits in mice have been shown by X-ray microanalysis to contain predominantly calcium (61%), phosphorus (29%) and much smaller quantities of iron, copper, zinc and magnesium. Yanai T. et al 1987, Jap. J. Vet. Sci. 49, 920-922.
Incidental Hippocampal Epidermoid Cyst

- Epidermoid cysts, blue arrow, may locate in midline cerebral locations as well as meningeal, spinal and radicular positions
- Note the distortion of habenular nuclei and hippocampus
- This example is more cellular than many
Neuron necrosis
Thalamic Infarct from Arteriolar Embolization

Regional pallor signifies early necrosis of the affected area that involves much of the thalamus, hypothalamus, basis pedunculi and optic radiation unilaterally. In ischemic brain lesions, typical morphological criteria of neuronal necrosis may take in excess of 10-24 hours survival to manifest that appearance in H&E sections.

Note the blue arrow, left, and this inset image of the inciting fat and fibrin embolus.
Cortical infarct from arteriolar thrombosis

- This is a temporally more advanced response to infarction
- Note the gliosis (NOS)
- There is prominent capillary hyperplasia, blue arrow left, (AKA neovascularization, both terms are allowable in NTP Tox data management, TDMSE)
- A small arteriole, black arrow, at the pia is thrombosed
Multiple sites of brain necrosis

- Bilateral regional vulnerabilities of sites in rat brain here are due to the effect of inhaled carbonyl sulfide gas
- Dose 600 ppm 6 hr/day for 2 days
- Parietal cortex Area 1 (blue arrow) Thalamus (white arrow) Retrosplenial cortical necrosis (black arrow).
- Exposure reduces Regional Rat Brain Cytochrome Oxidase levels by as much as 55%
Cerebellar internal granular cell necrosis

- Acute necrosis of small neurons such as the granule cells of the olfactory bulb, dentate hippocampal gyrus, the internal granule cell layer of the cerebellum etc., are characterized by basophilic nuclear pyknosis with H&E staining.
- Necrotic nuclei (blue arrows) Normal (red arrows)
- Such important lesions are frequently missed at low power inspection
- Note that some subjacent Purkinje cells have basophilic artifact.
Pyriform cortical neuronal necrosis
Commonly overlooked at low power examination.
Hippocampal dentate neuronal necrosis
Cerebellar Purkinje cell acute neuronal necrosis

- This eosinophilic neuronal lesion (blue arrow) is commonly the result of ischemia
- It may also result from any influence that impairs neuronal energy metabolism
- Note the necrotic glial nucleus (black arrow)
- The red arrow indicates a relatively normal Purkinje cell for comparison
Olfactory bulb neuronal necrosis & reactive swelling

- Acute necrosis of internal granule cells of the olfactory bulb
- Note that the change is worst on the left (blue arrow)
- There is loss of neurons as well as swelling & rarefication of the entire bulb
- This is partly due to reactive fluid accumulation in the neuropil
Hippocampal neuronal mineralization

- Neuronal necrosis of hippocampal pyramidal cells in the CA-2 region
- There is advanced ferrugination of neurons (blue arrows)
- Representing dystrophic mineralization with Ca, P & Fe)
- This indicates the chronicity of the neuronal necrotic process
Cerebellar internal granule cell mineralization

- Calcospherites deposited in a region of former cerebellar internal granule cell necrosis
- Note the characteristic concentric mineralized lamellated structures (blue arrow)
Brain white matter lesions
Cerebellar intramyelinic “edema”

Left, note the effect of hexachlorophene intoxication and on the right the normal appearance for comparison. Cresyl violet for neurons and LFB counterstain for myelin. Four images courtesy Dr. G. Krinke
On the left, note the intense spongiform effect of hexachlorophene intoxication and on the right, normal for comparison. Importantly, there is severe spongiform change of white matter without any evidence of myelin breakdown or cellular reaction. The phenomenon may be reversible. Cresyl violet for neurons and LFB counterstain for myelin
Cerebellar axonopathy

- Eosinophilic spheroids and oval-to-elongated structures (blue arrows)
- These represent injured axons which when sectioned in various planes affect their appearance.
Spinal and peripheral nerve axonal injury
Spinal cord axonopathy

- Note the distended zone of myelin sheaths and the enclosed swollen axons.
- Longitudinal or tangential sections may show discontinuous swollen, tapered, profiles of these injured axons.
Cervical cord axonopathy

- Note the spinal funicular swollen axon, a so-called spheroid (blue arrow).
- The pyknotic macrophage in a vacuole (white arrow). Represents a later stage of axonal injury characterized by axonal phagocytosis.
- Seldom in spinal and nerve axonal phagocytosis does the macrophage appear viable.
- Other vacuoles in the tissue probably represent sites where axons have been totally resorbed.
Murine bilateral cervical cord demyelination

- Bilateral demyelination & cavitation of the lateral funiculi (blue arrows)
- Mouse, spinal cord, paraffin section, Hematoxylin & Eosin (H&E) stain
- This lesion was attributed to infection with an atypical Theiler’s Murine Encephalomyelitis Virus (TMEMV) strain.
- Note that the term demyelination is restricted to processes of myelin loss with relative preservation of axons
- TMEM Virus is seldom if ever encountered in North American laboratory mice
- Two images courtesy Dr. G. Krinke.
Sciatic nerve demyelination

Sciatic nerve, paraffin section, Hematoxylin & Eosin (H&E) stain, myelin digestion chamber containing an intact axon between arrow heads. From the same study as the previous Figure.
Sciatic nerve axonopathy

- Sciatic nerve with a typical appearance of axonopathy
- Commonly called Wallerian degeneration.
- Note the digestion chamber and axonal fragmentation (Blue arrow)
Normal thoracic dorsal root ganglion

Cresyl violet stain

Note the clear cytoplasmic region, white arrow, representing the axon hillock that normally is relatively devoid of Nissl substance. Two images courtesy Dr. G. Krinke
Neuronal vacuolation, blue arrow, while it may be seen in any population of neurons undergoing degeneration, is particularly a concern for its neuropathological significance in root ganglia.

It may appear there as an incidental finding especially in aged animals but this finding needs close consideration before being discounted.

This image is the genuine lesion of pyroxidine-induced cytoplasmic vacuolation of root ganglion cells.
Cellular reactions to injury
Gliosis (NOS) and capillary hyperplasia

- Reactive gliosis (NOS), white arrows, and capillary hyperplasia, blue arrow, in a site of former brain injury.
- Capillaries are quickly responsive to injury of adjacent tissue.
- Endothelial nuclear hypertrophy is evident within 12-24 hours.
- Early reactive astrocytes have swollen clear nuclei.
- If required in a study the specific identity of cells in gliosis demands special techniques.
Capillary endothelial hypertrophy
At 72 hours post-infarction rat cortex

- Note the reactive endothelial hypertrophy, blue arrow.
- This feature, at 12-24hrs, is often the first morphological indication of injury to the brain.
- It may be observed at low magnification in an affected region.
- Here at 72 hours macrophage presence, and some fragmentation of the affected tissue is also evident.
Microgliosis and mononuclear perivascular cuffing

- Microgliosis is shown adjacent to an inflammatory mononuclear perivascular cuff, blue arrow.
- In any neural injury as many as 40% of infiltrating reactive cells have peripheral blood origin.
- Note the many elongated, irregular nuclei of typical microglial cells.
Microgliosis

- Here are shown several activated microglial cells (rod cells) as they migrate under chemotactic stimuli to a region of neural injury. H&E stain
- It is not possible to say whether these cells are resident microglia or those derived from infiltrating monocytic cells, carried to the nervous tissue by the blood stream.
Rat microglial cells stained brown with Iba-1 IHC

- This technique identifies their microglial stellate processes and in this case their quiescent round nuclei.
- Ionized calcium binding adapter molecule 1 is a 17-kDa EF hand protein that is specifically expressed in macrophages/microglia.
- Note the other unstained glial cells (blue arrows) and neuron (white arrow).
- The rowed nuclei probably represent oligodendroglial cells; the other one presumptively an astrocyte.
Lipid phagocytes (Gitter cells) in a zone of cortical infarction, 72 hrs duration

- Note the well-formed macrophages, blue arrows, commonly present 48 hours after advent of brain necrosis.
Hemosiderin-laden macrophages

- Multiple brown pigmented hemosiderin-laden macrophages, blue arrow at a site of former hemorrhage in the corpus callosum.
- It is important to recognize that the corpus callosum normally has an apparent increase in cells particularly those of rowed oligodendroglial nuclei.
Hippocampal degeneration with macrophages and crystalline cholesterol deposits

- Mature degenerate focus, blue arrow
- Reactive gliosis (NOS), macrophages and crystalline-shaped cholesterol clefts are present
- The lesion is adjacent to the dentate gyrus of the hippocampus.
- Such a lesion might be part of a treatment related finding or incidental
Gemistocytic astrocytosis

- Reactive gemistocytic astrocytosis in a site of healing neural injury stained with H&E.
- One might date this lesion as about 12-14 days post injury
- Note the typical eccentric nucleus and prominent eosinophilic cytoplasm of these reactive astrocytes, blue arrows
- Such cells would stain strongly positive with gliofibrillary acidic protein (GFAP)
- There is also a less frequent micro-binucleate form, black arrow
The rationale for requiring additional & improved neural sections

• Societal concern for rising incidence of mental dysfunction in young and old
• A mounting demand for evaluation of potentially, and known, neuroactive compounds
• A need for assessment of brain regions beyond those found in the 3 sections normally examined

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