Introduction to the Toxicologic Pathology of the Nervous System

Katie Kelly Brennan, DVM, PhD, DAVCP
kkelly12@jhmi.edu

Objectives

• Understand the basic anatomy of the CNS & PNS
  – Sampling methods & techniques
• Be able to list the cell types and their functions
• Understand the basic pathology of the nervous system
• Be able to list the mechanisms of neurotoxicity
  – Be able to discuss specific toxicants and their effects

Overview

• Nervous system anatomy and histology
  – CNS
  – PNS
• Basic pathology of the nervous system
• Mechanisms of neurotoxicology

Basic Organization & Histology

The Nervous System

• Central Nervous System
  – Integrating, processing, coordinating sensory data and motor commands
  – Intelligence, memory, learning and emotion
  – Made up of the brain and the spinal cord
• Peripheral Nervous System
  – Provides sensory information to the CNS
  – Carries motor commands from the CNS to peripheral tissues and systems

The Central Nervous System (CNS)

• Brain
  ▪ Cerebrum
    ▪ gyri (out) and sulci (in)
    ▪ Basal ganglia
  ▪ Diencephalon/Thalamus
  ▪ Cerebellum
  ▪ Brain stem
    ▪ Midbrain
    ▪ Pons
    ▪ Medulla
  ▪ Spinal Cord
CNS Organization

- Grey matter
  - 100s of diverse neuroanatomic regions
  - Neuron cell bodies
  - Neuropil
    - Dendrites
    - Unmyelinated nerve fibers
- White matter
  - Primarily myelinated nerve fibers with glial cells
- Meninges
  - Dura Mater
  - Arachnoid/leptomeninges
  - Pia Mater
  - Cerebrospinal fluid circulates in ventricular system
    - produced by choroid plexus
CNS cell types

- Brain
  - Neurons
  - Neuroglia
    - Oligodendrocytes
    - Astrocytes
    - Microglia
    - Ependymal cells
- Meninges

Neurons

- Wide variety of shapes and sizes
- Granular basophilic cytoplasm- Nissl
- Large nuclei
- Cell processes
  - Axon
  - Dendrite
- Prominent nucleoli

Astrocytes

- Most numerous glial cells
- Long branched processes
  - Contact/support neurons, neuronal processes-provide structure
  - Cover capillary basement membranes- blood brain barrier
  - Control brain microenvironment
  - Cover basement membrane of pia mater
  - Role in guiding development
  - Limited repair
- IHC/heavy metal impregnation techniques required to see processes

The Blood-Brain Barrier

- Formed by endothelial cells, basement membrane, & astrocyte foot processes
- Immune interface
- Controls CNS homeostasis
  - Cell trafficking
  - Metabolite transport
  "blocks bugs and drugs"
Oligodendrocytes

- Responsible for myelination of axons in CNS
- Flattened concentric layers of plasma membrane wind around axons
  - Myelin(fat) - electrically insulating
  - Cooperativity in formation of myelin sheath around axons
- Small, rounded condensed nuclei
- Cytoplasm unstained by H&E

Microglia

- Part of monocyte-macrophage system
- Difficult to ID by conventional processing

Ependyma

- Epithelial lining of ventricles and spinal canal
- Cuboidal/low columnar cells
- Ciliated
- No basement membrane

Specialized Ependymal Cells- Choroid Plexus

Sampling the CNS

- High throughput-parasagittal section of brain + hemi-coronal section from the contralateral half
- Routine standard toxicology studies- coronal sections
- Additional sectioning for suspected neurotoxin/ uncertain specific CNS target sites
  - perfusion fixation
- Exsanguination, careful brain extraction, gentle manipulation
Processing the mouse brain

- Coronal/cross sections of head
Collection of Spinal Cord

Jordan et al. Preparation and Analysis of the Central Nervous System

The Peripheral Nervous System

- Subdivided into
  - Afferent nervous system
    - brings the sensory information to the CNS
  - Efferent nervous system
    - carries motor commands to muscles or glands
  - Somatic nervous system - controls skeletal muscle contractions
    - Under conscious control (voluntary)
  - Autonomic nervous system - regulates smooth muscle, cardiac muscle, and glandular activity at subconscious level (involuntary)
    - Parasympathetic
    - Sympathetic

PNS Structure

• Spinal roots
  • Ganglia
    • grouping of PNS neuron cell bodies
  • Nerves and branches
    – Cranial
    – Spinal
    – Peripheral
• Enteric Nervous System
• Sensory Receptors
Jortner, BS. Preparation and Analysis of the Peripheral Nervous System.

**PNS supporting cells**

**Neuroglia**
- **Satellite cells**—regulate exchange of nutrients and waste products
  - surround neuron cell bodies in ganglia
- **Schwann cells**—myelinate axons from PNS neurons
  - direct regeneration of damaged axons

**Peripheral Nerve Organization**

**Normal Structure of Peripheral Nerve**

**Sampling the PNS**
- Obtain samples from proximal and distal nerve levels
- Perfusion fixation preferred
  - EM: 2.5–3% glutaraldehyde
  - Paraformaldehyde (1–4%) is better for IHC, but it does not preserve myelin well
  - a small amount of glutaraldehyde (0.1–0.2%) will help preserve myelin
- Epoxy resin embedding—better resolution vs. H&E
- Morphometry, nerve fiber teasing
Histomorphology of Neuronal Damage/Death

- Neuronal necrosis
  - Stains/autofluorescence
- Chromatolysis
- Neuronophagia

“Dead Red” Neurons

Detecting Neuronal Degeneration

Chromatolysis

Satellitosis/Neuronophagia

Responses of Glial Cells

- Astrocytosis
- Myelin vacuolation
- Gliosis
- Gitter cells
- Inflammation
Astrocyte Activation

Alzheimer Type II Astrocytes

Myelin vacuolation/edema

Gliosis/Glial Nodules

Demyelination

Demyelination/Gitter Cells
Histomorphology of Injury to Axons

- Wallerian degeneration
- Spheroid formation
- Secondary demyelination
- Bands of Büngner

Digestion chambers

Spheroids

Neurotoxicology basics

- Adverse structural (neuroanatomical) or functional (neurochemical, physiological, or behavioral) effects on the nervous system following xenobiotic exposure during development and in maturity
  - CNS
  - PNS
  - Nerve endings
  - Effector organs
Neurotoxicology basics, cont
• Predisposed to injury due to complexity, limited repair capacity
• Functional/anatomic interrelatedness of distant parts
• High metabolic rate, blood flow
  – Glucose dependence
• High lipoprotein content
  – Oxidative injury, lipid peroxidation
  – Absorption of lipophilic compounds

Mechanisms of Neurotoxicity
• Neuronopathies
• Axonopathies
• Myelinopathies
• Toxicants affecting BBB integrity
  – Astrocyte targets
  – Vascular
• Neurocarcinogens
• Developmental neurotoxicants

Manifestations of Toxicologic Agents

Neuronopathies
• Toxicants targeting the neuron
  – Trimethyltin (TMT)
  – Metals
    • Manganese, methyl mercury, aluminum, lead, zinc toxicity/hypoCu
  – Drugs
    • 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
    • Chemotherapeutics
      – Doxorubicin, vincristine, cisplatin
  – Toxins
    • Clostridial toxins
    • Locoweed, yellowstar thistle
  – Neurotransmitter analogs
  – Nutritional
    • Pyridoxine (vitamin B6)

Clostridial Toxins
• Botulinum toxins produced by Clostridium botulinum
• Progressively increasing flaccid paralysis
  – Death due to respiratory failure
• Toxins cause retention of presynaptic vesicles @ cholinergic synapses

Botulism
Domoic Acid
- Glutamate analog produced by algae & diatoms that accumulates in shellfish
  - Amnesic shellfish poisoning
  - Neurologic disease in marine mammals
- Excitatory & cytotoxic effects on hippocampal neurons

Vitamin B₆ injury to DRG neurons (PNS)

Axonopathies
- Axon is the primary site of toxicity=chemical transection
  - β,β'-iminodipropionitrile (DPN)
  - Acrylamide
  - Carbon disulfide
  - N-hexane
  - Chemotheurapeutics
    - Vincristine
    - Organophosphates

Organophosphate Axonopathy

Myelinopathies
- Myelin destruction
  - Direct myelin damage
  - Toxicity to myelin producing cells
- Triethylin
- Bromethalin rodenticide
- Tellurium

Tellurium Myelinopathy
**Toxicants affecting BBB integrity**

- Increase vascular permeability
  - Polioencephalomalacia
    - Thiamine deficiency
      - Thiaminase containing foodstuffs
    - Lead, sulfur, or intoxicosis
  - Leukoencephalomalacia
    - Fumonisin mycotoxin

**Polioencephalomalacia**

- In carnivores, edema, vascular dilation, hemorrhage + neuronal necrosis of deep cortical grey matter/nuclei of cerebellum & brainstem
- Role for astrocytes?
- Different lesions in ruminants, consisting of cerebral edema & laminar necrosis of cerebral cortical grey matter
- Thiamine (Vitamin B12) is an important cofactor for enzymes involved in carbohydrate metabolism

**Neurocarcinogens**

- Genotoxins
  - Acrylonitrile
  - Methyl- and ethyl-nitrosurea (MNU & ENU)

**Developmental Neurotoxicants**

- Toxicants can induce overt malformations, histologic lesions, or persistent behavioral changes
  - Methanol/ethanol
  - Plant toxins
- Variation in timing of neuronal development & myelination

**Plant teratogen cyclopamine**
Cyclopamine as Anticancer agent

- Cyclopamine exerts teratogenic effect by inhibiting Hedgehog (Hh) pathway activation
  - Regulates growth & migration of neural progenitors
  - Mutations causing Hh activation seen in medulloblastomas
- Potential therapeutic agent treatment of medulloblastoma

Questions??