THE RETINA
A VULNERABLE, SENSITIVE and PREDICTIVE TARGET of TOXI

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Outline of Presentation

◆ Introduction to retinal anatomy, biochemistry, physiology, toxicology and vulnerability
◆ Environmental toxicants and toxins: Sources & ADME
◆ Environmental toxicants and toxins: Common signs and symptoms of visual system dysfunction
◆ Four principles learned from retinal toxicology studies with examples

10 Excellent Reasons To Study The Retina

➢ Retina is a major target site of drugs and toxicants
➢ Most essential features are similar in humans, non-human primates and rodents
➢ Structure-function relations are well-established
➢ Contains numerous types of neurons and glia with a wide diversity of synaptic transmitters and second messengers
➢ Neurogenetic steps of development are known for most neurons
➢ Can be studies in vivo and in vitro with behavioral, biochemical, cell/molecular biology and pharmacological techniques
➢ Easily accessible
➢ Numerous well characterized transgensics, knock-outs and knock-ins available
➢ Model of the CNS
➢ Use of ocular fluids and retina in postmortem analysis
Retina is Especially Vulnerable to Toxicant-Induced Alterations in Structure and Function

- Highly vascularized: dual circulatory supply
- Highest rate of metabolism and oxygen consumption of any tissue in the body: especially the photoreceptors
- Large number of complex synaptic pathways mediating graded and action potentials: no redundancy
- Presence of melanin in RPE: binding depot site
- Each retinal layer can undergo specific as well as general toxic effects.
- The alterations/deficits include visual field deficits, decreased color perception, night blindness, retinal hemorrhages and vasoconstriction and macular edema.

Schematic Diagram of Eye with Enlargement of Retina

The Adult Mammalian Retina Contains Six Types of Neurons, a Müller Glial Cell and a Dual Vascular Supply

Adapted from Contini and Raviola, PNAS 2003
The High Rate of Retinal Oxygen Consumption Creates a Borderline Hypoxia & Increases Vulnerability

Adapted from Linsementier, J Gen Physiol 1986
Adapted from Medrano and Fox, Exp Eye Res 1995
Dark- and Light-Adapted Rod and Cone Photoreceptors Have Different Functional and Bioenergetic Properties

Adapted from Perkins et al., Molecular Vision 2003; Johnson et al., Molecular Vision 2007

![Diagram of photoreceptors]

Rod ON, Cone ON and Cone OFF Bipolar Cell Pathways

Sharpe and Stockman, TINS 1999

![Diagram of bipolar cell pathways]

Sources of Environmental Toxicants and Toxins

![Images of various sources of environmental toxicants and toxins]
### Distribution of Ocular Xenobiotic Phase 2 Enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Tears</th>
<th>Corneal Iris/Conjunctiva</th>
<th>Retina</th>
<th>Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II reactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glutathione-S-transferase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sulphatransferase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>UDP-glucuronosyl transferase</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-acetyltransferase</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Note: "+" and "-" indicate that the enzyme was present/localized by immunohistochemistry, immunogold electron microscopy, Western blot or gene expression or absent, respectively, in human, monkey or rodent tissues.

**Fox and Boyes, Casarett and Doull's Toxicology 2013**

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### Common Signs and Symptoms of Visual System Dysfunction: Retina

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<th>Possible Pathophysiological Basis</th>
<th>Examples of Chemicals Producing this Effect</th>
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<tr>
<td>Reduced contrast sensitivity and visual acuity</td>
<td>Optic neuritis and/or degeneration of the optic tract, generally affecting retinal ATP production</td>
<td>Higher level chronic exposure to organic solvents such as carbon disulfide or hexane, ethambutol, diethylene glycol, ethylene glycol, isoniazid, theophylline, methanol, and bilirubin</td>
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<td>Non-ischemic anterior ischemic optic neuropathy</td>
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**Fox and Boyes, in Casarett & Doull's Toxicology: The Science of Poisons 2013**

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### Common Signs and Symptoms of Visual System Dysfunction: Optic Nerve/Tract, LGN and Visual Cortex

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PRINCIPLE #1.
THE DEVELOPMENTAL AGE DURING TOXICANT
EXPOSURE MEDIATES THE EFFECT
aka THE ALICE IN WONDERLAND EFFECT

Human Prenatal and Postnatal Organ Development

Retinal Neurogenesis Proceeds in a Fixed Histogenic Order in All
Mammalian Species: Early-Born and Late-Born Retinal Cells

Adapted from Altshuler et al., Critical Periods in Development, OCHP

Young 1985; Marquardt & Gruss 2002; Marquardt 2003
The Period of Developmental Lead Exposure Determines the Long-Term Changes in ERG Amplitudes and Retinal Cell Numbers

POSTNATAL: SUBNORMAL ERG & ROD APOTOPSIS
PRENATAL: SUPERNORMAL ERG & ROD / BIPOLAR CELL PROLIFERATION

Sir John Tenniel, Alice Wonderland 1865

Led Exposure to Post-Mitotic Neurons Produces Persistent Rod-Selective Scotopic Deficits, ERG Subnormality and Apoptosis

Lead Workers Have Rod-Selective Vision Deficits and Visual-Motor Alterations: Early Translational Studies
Postnatal Lead Exposure Produced a Cytochrome c- and Caspase 3-Dependent Rod-Selective Apoptosis Mediated by Bax Translocation to Mitochondria

The Period of Developmental Lead Exposure Determines the Long-Term Changes in ERG Amplitudes and Retinal Cell Numbers

Humans and Animals With Gestational Lead Exposure Exhibit Rod-Mediated ERG Supernormality and Retinal Proliferation


Gestational Lead Exposure Selectively Increased the Number of Rod Photoreceptors and Bipolar Cells

Giddabasappa et al. EHP 2011

Gestational Lead Exposure Increased the Number of Rhodopsin-Positive Rods and PKCα-Positive Rod Bipolar Cells in Adult Mice

Giddabasappa et al. EHP 2011

PRINCIPLE #2.
THERE ARE TISSUE, CELLULAR AND REGIONAL DIFFERENCES IN VULNERABILITY
aka Location, Location, Location
Retina vs Kidney: Tissue
Different Na+,K+-ATPase Low and High Ouabain-Affinity Isozymes Determined the Effects of Lead Exposure

![Graph showing tissue Na+,K+-ATPase activity levels in control, low lead, and moderate lead conditions.]

Rod, But Not Cone, Photoreceptors Are Selectively Affected by In Vitro or Postnatal Lead Exposure: CELL

Heavy Metals Affect Rod, But Not Cone, Photoreceptors
Donald A. Fox and Arnold J. Stillman

Rods are selectively altered by lead: I. Electrophysiology and biochemistry.

Rods are selectively altered by lead: II. Ultrastructure and Quantitative histology.

The Lead-Induced Loss of Rods Is Greater in the Central than Peripheral Retina and in the Inferior than the Superior Retina: REGIONS

In lead-exposed rats, there is a greater loss of rods in the posterior (central) compared to peripheral retina and in the superior compared to the inferior retina.

<table>
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<tr>
<th>Region</th>
<th>Control</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior retina</td>
<td>Superior</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Inferior</td>
<td>100</td>
</tr>
<tr>
<td>Peripheral retina</td>
<td>Superior</td>
<td>100</td>
</tr>
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<td></td>
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<td>100</td>
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* The rod and cone ratios remain at basal levels of 30:100 for length:18:1000 for width as in normal and 40 lead-exposed rats as described in the Materials and Methods.

† Values are given as the mean ± S.E.M. per 100 µm of retina.

‡ Significantly different from normals at P < 0.01.
PRINCIPLE #3.
CELL-CELL INTERACTIONS DETERMINE THE OUTCOME OF THE EXPERIMENT
aka LIVE FREE OR DIE HARD

The Presence/Absence of RPE With Cultured Developing Retina Determines the Phenotypic Effects of Exogenous Chemicals:
Important Implications for Drug & Toxicant Screening

Retinoic Acid with RPE:
Rod Proliferation, No Apoptosis, No Caspase Activation

Retinoic Acid without RPE:
Rod-Selective Apoptosis & Caspase Activation

Söderpalm et al., Investig Ophthalmol Vis Sci 2000

PRINCIPLE #4.
TOXICANTS and TOXINS CAN BE THE PERFECT TOOL FOR DECIPHERING MECHANISMS OF ACTION
aka THE RIGHT TOOL FOR THE RIGHT JOB
**Toxins As Tools**

- Tetrodotoxin (pufferfish) – inhibits Na⁺ channel
- Conotoxins (snails) - inhibit voltage-dependent channels
- Epibatidine (insects/poison dart frog) – AChM and AChN receptor agonists
- Batrachotoxin (melyrid beetles/poison dart frog) – activates Na⁺ channels
- Curare (plant) – AChN receptor antagonist
- Domoic acid (red algae) – kainic acid analog

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**Thank you!**