THE RETINA
A VULNERABLE, SENSITIVE and PREDICTIVE TARGET of TOXICITY

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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 studied in vivo and in vitro with behavioral, biochemical, cell/molecular biology and pharmacological techniques
- Easily accessible
- Numerous well characterized transgenics, 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

http://www.webvision.med.utah.edu/
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
Light Micrographs of Mammalian Central Retina

HUMAN

- Retinal Pigment Epithelium
- Rod & Cone Outer Segments
- Rod & Cone Inner Segments
- Outer Nuclear Layer
- Outer Plexiform Layer
- Inner Nuclear Layer
- Inner Plexiform Layer
- Ganglion Cell Layer
- Nerve Fiber Layer & Muller End Feet

RAT

Fox and Chu, Exp Eye Res 1988

http://www.webvision.med.utah.edu/
Electron Micrographs of Adult Mouse Outer Retina

(Perkins et al. Mol Vis 2003; Johnson et al. Mol Vis 2007)
The High Rate of Retinal Oxygen Consumption Creates a Borderline Hypoxia & Increases Vulnerability

Adapted from Linsenmeier, 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
Rod ON, Cone ON and Cone OFF Bipolar Cell Pathways

Sharpe and Stockman, TINS 1999
Sources of Environmental Toxicants and Toxins
Ocular Absorption and Distribution of Chemicals Following the Systemic Routes of Exposure

SYSTEMIC ROUTE OF DRUG and CHEMICAL EXPOSURE
Oral, Inhalation, Dermal, Parenteral

Retinal Choroid
Inner Retina

BLOOD

Optic Nerve, Brain & Other Organs

Corneal Endothelium
Aqueous Humor
Iris & Ciliary Body
Vitreous Humor
Lens

Fox and Boyes, Casarett and Doull’s Toxicology 2013
Ocular and Retinal Blood-Tissue Barriers

Yasukawa et al., 2006
## Distribution of Ocular Xenobiotic Phase 1 Enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Tears</th>
<th>Cornea Iris/Ciliary</th>
<th>Lens</th>
<th>Retina</th>
<th>Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1 reactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholinesterase (AChE)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alcohol dehydrogenase</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>Aldehyde dehydrogenase</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aldehyde reductase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Aldose reductase</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Carboxylesterase</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Catalase</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cu/Zn superoxide dismutase</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-/-</td>
<td>+</td>
</tr>
<tr>
<td>MAO-A or MAO-B</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CYP1A1 or CYP1A2</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>CYP1B1</td>
<td></td>
<td></td>
<td></td>
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<td>+</td>
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<tr>
<td>CYP2B1 or CYP2B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>CYP2C11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>CYP3A1</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CYP4A1 or CYP4B2</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CYP27A1</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
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</tr>
</tbody>
</table>

**KEY:** "+" 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.
## Distribution of Ocular Xenobiotic Phase 2 Enzymes

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>TEARS</th>
<th>CORNEA IRIS/CILIARY</th>
<th>LENS</th>
<th>RETINA</th>
<th>CHORIOID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase II reactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glutathione- S-transferase</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sulfotransferases</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>UDP-glucuronosyl transferases</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>N-Acetyltransferase</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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Fox and Boyes, Casarett and Doull’s Toxicology 2013
# Common Signs and Symptoms of Visual System Dysfunction: Retina

<table>
<thead>
<tr>
<th>Common Signs &amp; Symptoms</th>
<th>Possible Pathophysiological Basis</th>
<th>Examples of Chemicals Producing this Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>RETINA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor night (scotopic) vision and impaired dark adaptation</td>
<td>Damage to and apoptosis of rod photoreceptors &lt;br&gt;Acetylcholinesterase inhibitors</td>
<td>Lead, methyl mercury, vigabatrin &lt;br&gt;Organophosphate and carbamate insecticides; nerve gas agents</td>
</tr>
<tr>
<td>Altered color perception, central scotoma</td>
<td>Inhibition of cone photoreceptor sodium-pumps</td>
<td>Digitalis/digoxin</td>
</tr>
<tr>
<td>Altered color perception</td>
<td>Inhibition of cone photoreceptor cGMP-phosphodiesterase</td>
<td>Sildenafil and tadalafil</td>
</tr>
<tr>
<td>Impaired color discrimination (Blue/Yellow)</td>
<td>Damage to cone photoreceptors and inner retina</td>
<td>Chronic exposure to styrene and organic solvents; trimethadione; chronic high dose antibiotics</td>
</tr>
<tr>
<td>Impaired color discrimination (Red/Green)</td>
<td>Acquired damage to cone photoreceptors, neural retina and/or afferent visual pathway</td>
<td>Higher level chronic exposure to organic solvents, carbon disulfide or hexane; chronic carbon monoxide, chronic alcoholism, ethambutol</td>
</tr>
<tr>
<td>Loss of peripheral vision</td>
<td>Degeneration of peripheral retina and nerve fiber layer</td>
<td>Methyl mercury, vigabatrin</td>
</tr>
<tr>
<td>Reduced contrast sensitivity and visual acuity</td>
<td>Degeneration of the retinal ganglion cells and optic tract; microaneurysms and retinal vasculopathy</td>
<td>Acrylamide, carbon disulfide,</td>
</tr>
</tbody>
</table>
# Common Signs and Symptoms of Visual System Dysfunction: Optic Nerve/Tract, LGN and Visual Cortex

<table>
<thead>
<tr>
<th>Common Signs &amp; Symptoms</th>
<th>Possible Pathophysiological Basis</th>
<th>Examples of Chemicals Producing this Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPTIC NERVE and OPTIC TRACT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced contrast sensitivity and visual acuity</td>
<td>Optic neuritis and/or degeneration of the optic tract: generally affecting mitochondrial ATP production</td>
<td>Higher level chronic exposure to organic solvents such as carbon disulfide or hexane; ethambutol, ethylene glycol, isoniazid, linezolid and chloramphenicol, methanol, vigabatrin</td>
</tr>
<tr>
<td>Monocular and/or binocular visual loss</td>
<td>Non-arteretic anterior ischemic optic neuropathy</td>
<td>Amiodarone; sildenafil and tadalafil</td>
</tr>
<tr>
<td><strong>LATERAL GENICULATE and VISUAL CORTEX</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central scotoma</td>
<td>Degeneration of calcarine fissure of visual cortex</td>
<td>Methyl mercury</td>
</tr>
<tr>
<td>Visuomotor deficits and reduced contrast sensitivity</td>
<td>Visual and motor cortex dysfunction</td>
<td>Lead, chronic exposure to carbon disulfide, hexane and other solvents, toluene</td>
</tr>
</tbody>
</table>
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

Birth Order of Murine Retinal Cells

Retinogenesis

EARLY-BORN  LATE-BORN

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 APOPTOSIS

**PRENATAL**: SUPERNORMAL ERG & ROD / BIPOLAR CELL PROLIFERATION

*Sir John Tenniel, Alice In Wonderland 1865*

**Fox et al., Neurotoxicology 1991**

**Rothenberg et al., IOVS 2002**

**Sir John Tenniel, Alice In Wonderland 1865**
Lead Exposure to Post-Mitotic Neurons Produces Persistent Rod-Selective Scotopic Deficits, ERG Subnormality and Apoptosis

Fox DA, Farber DB.
Rods are selectively altered by lead: I. Electrophysiology and biochemistry.

Fox DA, Campbell ML, Blocker YS.
Functional alterations and apoptotic cell death in the retina following developmental or adult lead exposure.

He L, Poblenz AT, Medrano CJ, Fox DA.

He L, Perkins GA, Poblenz AT, Ellisman MH, Harris JB, Hung M. Fox DA.
Bcl-xL overexpression blocks bax-mediated mitochondrial contact site formation and apoptosis in rod photoreceptors of lead-exposed mice.
Effects of lead on the visual system of occupationally exposed subjects.

Williamson AM, Teo RK.
Neurobehavioural effects of occupational exposure to lead.

Jeyaratnam J, Boey KW, Ong CN, Chia CB, Phoon WO.
Neuropsychological studies on lead workers in Singapore.
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

POSTNATAL: SUBNORMAL ERG & ROD APOPTOSIS

PRENATAL: SUPERNORMAL ERG & ROD / BIPOLAR CELL PROLIFERATION

Sir John Tenniel, Alice In Wonderland 1865

Fox et al., Neurotoxicology 1991

Rothenberg et al., IOVS 2002
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 PKC\(\alpha\)-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

Fox et al., Toxicol Appl Pharmacol 1991
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. Sillman

Fox DA, Farber DB.
Rods are selectively altered by lead: I. Electrophysiology and biochemistry.

Fox DA, Chu LW.
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>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superior</td>
<td>Inferior</td>
</tr>
<tr>
<td>Posterior (central)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Rod nuclei remaining</td>
<td>100</td>
<td>83.1(\dagger)</td>
</tr>
<tr>
<td>% Cone nuclei remaining</td>
<td>100</td>
<td>96.2</td>
</tr>
<tr>
<td>Peripheral retina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Rod nuclei remaining</td>
<td>100</td>
<td>87.5(\dagger)</td>
</tr>
<tr>
<td>% Cone nuclei remaining</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* The rod and cone nuclei counts are based on 30 100-\(\mu\)m lengths; 10 consecutive lengths in each of three sections from 11 control and 12 lead-exposed rats as described in the Materials and Methods.

\(\dagger\) Values represent the mean \(\pm\) s.e.m. per 100 \(\mu\)m of retina.

\(\dagger\) Significantly different from controls at \(P < 0.01\).

Fox and Chu, Exp Eye Res 1988
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 without RPE: Rod Proliferation, No Apoptosis, No Caspase Activation

Retinoic Acid with 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
Thank you!