Unraveling Nicotine-Mediated Neurotoxicity Mechanisms: Translation is in the Tank

Part I

Robert L. Tanguay
Environmental Health Sciences Center
Environmental and Molecular Toxicology
Oregon State University

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Early Environmental Exposures And Adult Diseases

Focus on Embryonic Development

Why?

- Generally more responsive to insult... because
  
  Most dynamic life stage...and the full signaling repertoire is expressed and active, therefore fewer blind spots...
  Highest potential to detect interactions

- If a chemical or nanomaterial is developmentally toxic it must influence the activity of a molecular pathway or process... i.e. hit or influence a “Toxicity Pathway”

- Identify the immediate responses that lead to long lasting effects or disease
Why Zebrafish

- Share many developmental, anatomical, and physiological characteristics with mammals
- Molecular signaling is conserved across species
- Technical advantages of cell culture - power of in vivo
- Amenable to rapid whole animal mechanistic evaluations
- Outstanding model for human disease research

Human Disease Related Research in Zebrafish

- Parkinson's
- Schizophrenia
- Alzheimer's
- Inflammation
- Metabolic Disorders
- Lysosomal Diseases
- Leukemia
- Epilepsy
- Multiple Sclerosis
- Metastasis
- ALS
- Regeneration
- Cancers
- Obesity
- Sleep Disorders
- Deafness
- Blindness

Sleep Disorders

Cancers

Metabolic Disorders

Lysosomal Diseases

Epilepsy

Multiple Sclerosis

Metastasis

ALS

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Leukemia

Epilepsy

Multiple Sclerosis

Metastasis

ALS

Regeneration

Cancers

Obesity

Sleep Disorders

Deafness

Blindness
Complete Control Over Exposure Period

Examples from the Zebrafish Tool Box
Amenable to Rapid Assessments
small amounts of chemicals

384 well plate 4 embryos in Each well 23 hour embryo

Proof of Concept

- >65 million regular smokers in US as of 2007 (CDC)
- Nicotine is a drug of abuse, with apparent, adverse developmental consequences including cognitive and behavioral impairments
- Nicotine effects are mediated by nAChRs, a highly conserved family of ligand-gated ion channels in vertebrates
- nAChRs are critical for muscle and neuronal development, and axonal pathfinding
- The mechanism by which nicotine perturbs vertebrate development remains undefined.
Cigarette Smoking Prevalence US High School Females


Consequences Of Cigarette Smoking During Pregnancy

- Low birth weight
  Potential long term consequences
  - Premature deliveries
  - Infant mortality
  - Implicated in cognitive deficits
Nicotinic Receptors Mediate Nicotine Responses

• Multiple subunits (we have cloned all 28)
  – 8 alpha subunits – alpha 2-10
  – 3 beta subunits - 2, 3, and 4

• Different subtypes may regulate different physiological functions

• Alterations in expression of specific subtypes may play a role in certain pathologies

• Different subtypes display distinct pharmacology and channel properties

Structure OF nAChR Subunits
Can Zebrafish Be Used To Fill Information Gaps?

- What is the impact of transient nicotine exposure on CNS development and function?
- What receptor subunits mediate the responses?
- What mechanisms underlie the responses?
- What other cellular factors mediate the responses?
What are the nicotinic responses?

Altered Growth

Exposure 33 μM nicotine
22-66 hpf

hours post fertilization

control
66hpf

nicotine

42 66 120
Altered Behavior

CONTROL

NICOTINE (22 - 66 hpf)

72 hpf

Transgenic Animals– Islet1 GFP
Nicotine Impacts Spinal Neurons
Live Transgenic Imaging

CONTROL

42hpf

66hpf

NICOTINE

42hpf

66hpf

Exposure 22 - 42 hpf

Exposure 22 - 66 hpf

Islet-1 GFP

Motoneurons Are Not Deleted
Coupled Immunohistochemistry and GFP Imaging

66hpf

Exposure 22 - 66 hpf

Zn5 antibody and islet-1 GFP
Nicotine Delays GFP Expression
Live Transgenic Imaging

**CONTROL**

![CONTROL 66hpf](image1)

![CONTROL 120hpf](image2)

**NICOTINE**

![NICOTINE 66hpf](image3)

![NICOTINE 120hpf](image4)

*Islet-1 GFP*

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Branching Deficits Persistent
Live Transgenic Imaging

![Branching Deficits](image5)

*Exposure (22-66hpf)*
Path Finding Errors Resulting From Early Nicotine Exposure

CONTROL

NICOTINE

192 hpf

Exposure 22 - 66 hpf

Zn5 antibody

nAChR Antagonist Block Response
Live Transgenic Imaging

66hpf

con

66hpf

nicotine

66hpf

Nicotine + 100 nM MLA

66hpf

Nicotine + 2 μM MLA

MLA methyl-lyconitine (α7)
DHβE dihydro- β-erythroidine (α4β2)

66hpf

con

66hpf

nicotine

66hpf

Nicotine + 20 μM DHBE
Take Home Message

• Zebrafish as a non-mammalian vertebrate model offers a number of powerful advantages to rapidly dissect mechanisms

• Current focus:
  • Immediate to late gene expression changes in response to nicotine - whole animal, and specifically in target cells
    • mRNA, miRNA and Protein
  • Adult behavioral responses from transient developmental nicotine exposure