Case studies: Using the zebrafish to evaluate neurobehavioral phenotypes

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Neurobehavioral Diseases

- Anxiety Disorders
- Autism
- Alzheimer’s
- Social disorders
Zebrafish as an Animal Model

- Physiologically + Genetically homologous
- Large number of progeny
- High Throughput screens
- Cost and space efficient
- Complex emotional behavior
Research Goals

Stage 1  Embryonic

Stage 2  Adults

Micronutrient Deficiency

Chemical Exposure
Stage 1: Embryonic Photomotor Response (EPR)

- Dark adapted embryonic zebrafish response to light stimulus
  - 17 hpf
- Two stages
  - Stage 1 (before light)
    - Infrequent coiling of body axis
  - Stage 2 (after light stimulus)
    - Vigorous coiling for 5-7s
Zebrafish Hindbrain

- Embryonic Photomor Response
  - Not fully understood
  - Cells in Hindbrain
Photomotor Response Analysis Tool (PRAT)

- At least 24 hours post fertilization
- Movement index
  - Frame pixel differences
- Light cycles

<table>
<thead>
<tr>
<th></th>
<th>1st light pulse</th>
<th>2nd light pulse</th>
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<tbody>
<tr>
<td>Background</td>
<td>30 Seconds</td>
<td>40 Seconds</td>
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<tr>
<td>Excitatory</td>
<td></td>
<td></td>
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<tr>
<td>Refractory</td>
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Normal EPR Activity

[Graph showing movement over time with peaks at different times]
EPR Developmental Time Series

• Goals
  • Generate baseline
  • Change throughout development time?

• Time series
  • 9 am-10 pm
    • PRAT run every hour
Spawning Tanks
Embryo collection

Embryo Loading

96 well plate and incubated for 24 hrs

Photomotor Response Analysis Tool (PRAT)

0-1 hpf

6 hpf

6 hpf

24 hpf
EPR Movement Activity Chart
# EPR Movement Peaks

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<tr>
<th>Hours post fertilization</th>
<th>Time</th>
<th>Movement peak</th>
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<tr>
<td>25</td>
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<td>43.66</td>
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<tr>
<td>26</td>
<td>10 am</td>
<td>22.89</td>
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<tr>
<td>27</td>
<td>11 am</td>
<td>39.55</td>
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<td>28</td>
<td>12 pm</td>
<td>105.57</td>
</tr>
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<td>1 pm</td>
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<td>36</td>
<td>8 pm</td>
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<tr>
<td>37</td>
<td>9 pm</td>
<td>255.07</td>
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EPR Summary

- Optimal Times to use for EPR baseline?

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- Screen for hyperactivity or hypoactivity
- Values not too high or too low
Stage 1  Embryonic

Stage 2  Adults

Micronutrient Deficiency  Chemical Exposure
Stage 2: Micronutrient Deficiency

- May have many negative effects
  - Tissue damage
  - Damage to nervous system
  - Negative behavioral effects
Vitamin E

- Micronutrient
- Almonds, Bell peppers, Papayas
- Antioxidant
- Protects lipid membranes
Vitamin E Research Questions

• Does Vitamin E deficiency have a negative effect on learning and memory in adult zebrafish?
• Does Vitamin E deficiency affect startle response?
Vitamin E Diets

54 days old

Commercial Feed

Vitamin E Diets

Shuttlebox Startle response

90 days old
Shuttlebox Design

- **Center divider:** forces fish under opening at bottom for detection by thru beam sensor.
- **Black acrylic shuttle box**
- **LED Light Bar:** one on each end.
- **LED optical window**
- **Thru beam optical window**
- **Stainless plate for shock delivery:** One at each end of shuttle box.
- **Thru beam transmitter/receiver lens mounts:** two side-by-side.
- **Thru beam Keyence transmitter/detector amplifiers and fiber optic leads to lenses**
- **Shuttle indicators, and shock PWM board (upper) under protective acrylic shield:** Arduino R3 controller with SMT power and USB jacks (lower).
Shuttlebox Protocol

- **Acclimation period**: 10 minutes
- **“Seek” period**: 8 secs
- **“Shock” period**: 16 secs
- **Rest period**: 60 secs

1 Trial

- Zebrasfish conditioned to associate dark side with shock
- Learn to swim to the light side
- 30 consecutive trials
- Fault out Feature
Vitamin E: Shuttlebox Results

- Time to Accept Side (TimetoASide)
- Initial decision time
- Each fish fit with linear regression
- P value of 0.00338
- Effect on learning
Vitamin E: Startle Response

- Reflex invoked by unexpected Stimulus (Tap)
- Possible Threats
- Cognitive Processing of stimuli
- Can be used to study cognitive deficits
- Characterized in Adult Zebrafish
  - High velocity swimming
Startle Response Assay

- Taps generated using Solenoids
- Tracked with Noldus EthioVision XT

zebrafish Visual Imaging System (zVIS)
Startle Response Protocol

- Acclimation period: 10 min
- 5 min taps
- 5 min taps
- 5 min taps
Normal Startle Response
Startle Response in Vitamin E diet groups

![Graph showing total movement (cm) for different diet groups at Tap 1, Tap 2, and Tap 3. The graph compares E- and E+ diets, with asterisks indicating significant differences.]
Startle Response Assay Results

• Vitamin E- fish do not get desensitized to tap stimulus
  • Nervous system
Stage 1  Embryonic

Stage 2  Adults

Micronutrient Deficiency  Chemical Exposure
Stage 2: Chemical Exposure

• Lead to abnormal behavior
  • Social
  • Fear responses
  • Negative effect on learning
Benzo[a]pyrene
Research Question

• Does Benzo[a]pyrene Developmental Exposure cause transgenerational effects?
Three Generations of Fish

- Only F0 generation directly exposed

Behavioral effects seen in F0
  - Mainly used F1 and F2 due to availability
  - F0 for shuttlebox
Exposing zebrafish to Benzo[a]pyrene (BaP)

Dechorionated embryos

6 hpf

96 well plate

1.25 ppm BaP
2.5 ppm BaP
0.1% DMSO controls

Rinsed and raised

120 hpf
Shoaling a Social behavior

• Not Schooling
  • Coordinated swimming
• Form Close groups
• Predator Defense
• Increased mating opportunity
Why Shoaling Assay?

- Humans are social
- Neurodevelopmental Disorders
  - Anxiety disorders
  - Depression
  - Autism
- Zebrafish Homology
  - Brain layout
  - Brain neurochemistry
Shoaling Parameters

• Inter-individual Distance (iid)
  • Average distance between farthest fish

• Nearest Neighbor Distance (nnd)
  • Average distance between closest fish

• Polarization
  • Direction of shoal as a vector
  • Higher value = Higher disassociation
Inter-individual Distance (iid)
Nearest Neighbor Distance (nnd)
Polarization
Shoaling Results

• Based on iid, nnd, and polarization
  • Disassociation
    • F1 1.25 ppm BaP fish
    • F2 2.5 ppm BaP fish

• Some generational effects
  • Some recovery as BaP Values decrease between F1 and F2 generation
    • DMSO control values also decrease
      • May effect behavior
Fear Response

- Related to Fitness
- Natural Response to Predators
- Clinical Relevance
  - Neurobiological disorders
    - Exaggerated responses
Predator Video
Zebrafish Visual Imaging System

- 8 tanks per zVIS
- Tanks have clear side
- Video monitors
- Noldus EthioVis.
- Arenas
- Close zone
- Middle zone
- Far zone
Predator Close. Cumulative Duration

Time (seconds)

Average Duration (%)
Fear Response Results

• Generational effects seen between F1 and F2 2.5 ppm BaP fish
• Generational effects in F1 and F2 1.25 ppm BaP fish but not as strong
• Duration in far zone between F1 and F2 0.1% DMSO controls decreases
Benzo[a]pyrene Shuttlebox Results

• 0.1% DMSO fish learn quicker with each generation
• F0 2.5 ppm, 1.25 ppm BaP learn quicker than F0 controls
• No real significant generational effects
• Possible effect on learning
  • Non DMSO controls
Conclusion

**Embryonic Photomotor Response Study**
- Optimal Baseline EPR activity at 28-29 hpf

**Adult Vitamin E Study**
- Vitamin E+ learn quicker
- Vitamin E- don’t desensitize to Tap Stimulus

**Adult Benzo[a]pyrene Study**
- Possible trangenerational effects seen in Shoaling and Fear Response due to Benzo[a]pyrene exposure
- Possible effect on learning
- DMSO exposure may effect behavior
Future Directions

• High throughput screening of EPR phenotype
• Neuropathology tests on Vitamin E- fish
• Confirmation to see if the behavioral effects that were seen in the F2 generation are also in the F3 generation fish
• Using non-DMSO exposed fish in shoaling, fear response, and shuttlebox assays
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