Mixing Hazardous Agents in a Mouse Model: Noise with Lead and Cadmium
Outline

- Noise as a starting point
- Challenges in research and study design
- Study methods
- Results
- Conclusions
Effects of lead and noise exposures on hearing ability.

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Abstract
Lead and noise, via different mechanisms, may damage hearing ability, and, in some cases, cause severe hearing loss. This study explores possible independent and synergistic effects of lead and noise on auditory function. The authors conducted a study in two lead-battery manufacturing factories. Lead and noise were the two most common sources of occupational exposure. Blood lead level, ambient lead concentration, noise exposure level, and hearing thresholds of 339 lead-battery manufacturing workers were measured. The authors obtained demographics and working histories via an interview. A total of 220 lead-battery workers were exposed to high levels of lead and noise; their average blood lead level and their average noise exposure level was 86.0 dBALeq. Multivariate analysis, in which possible risk factors were considered, demonstrated a significant correlation between a high, long-term lead exposure index (defined as the ratio of ambient lead concentration) and decreased hearing ability. In contrast, such a correlation between short-term lead exposure and hearing ability was not significant. Furthermore, neither noise exposure level alone nor lead exposure level and short- or long-term lead exposure was correlated significantly with hearing ability. The study highlights the equally important, but typically ignored, issue: lead exposure might precipitate a more severe auditory than noise-exposure related hearing loss. This worrying effect requires that preventive measures be taken against noise exposure, which is as essential as lead exposure.
Noise

- Ground study in a known hazardous agent
- What are attributes of populations at risk?
- What agents commonly co-occur?
- What risk factors are carried by these populations?
- Can vulnerabilities can be replicated in the model?

Image: flickr.com/photos/92119884@N02/
Pathway to Hearing

Image: maineaudiology.org
Mouse Cochlea
The Cochlea

Image: hyperphysics.phy-astr.gsu.edu

Image: meniett.com.msb.medtronic.com
Outer and Inner Hair Cells

Anti-myosin VIIa-Diaminobenzidine stained cochlea at 400x
Challenges of Mixture Modeling

- Reviewing literature extensively
  - Toxicology
  - Epidemiology
- Postulating mechanisms for synergism
- Choosing animal and strain
Toxicology Pre-natal Lead Exposure in Mice:

- 0.1 mM and 2 mM in drinking water altered neuronal proteins within the auditory brainstem nuclei through increased phosphorylation and significantly affected auditory processing.

Lead as an Ototoxic Agent?

- **Occupational Epidemiology:**
  - Steel plant workers with BLL over 7 µg/dL have higher odds of hearing loss over 3 to 8 kHz (ORs between 3.06 and 6.26; p<0.05-p<0.005).
  

- **Environmental Epidemiology:**
  - Age-related hearing loss odds increase with heightened bone lead levels even after accounting for occupational noise exposure (OR = 1.48, 95% CI, 1.14-1.91).

Cadmium as an Ototoxic Agent?

- **Environmental Epidemiology**
  - Higher odds of hearing loss at low frequencies in highest cadmium exposed quartile, OR = 3.08 (1.02, 9.25).

- **Toxicology**
  - Threshold shifts in C57BL/6 mice after 30 days of 1 mM cadmium
ROS Damage Causes Hearing Loss

Postulated Mechanism

Lead or cadmium exposure interacts with noise exposure resulting in increased levels of hearing loss through generation of reactive oxygen species damaging cells in the inner ear and the auditory pathway.

Hearing Study Animals

- Monkey
- Guinea Pig
- Chinchilla
- Rat
- Mouse

Images: flickr.com
Users randya38, nationalzoo, saphirai, 121483302@N02

Image: criver.com
A Tale of Two Mice Strains

- **C57/BL6 mouse**
  - Accelerated age-related hearing loss beginning at 10 weeks

- **BALB/c mice**
  - Progressive high-frequency hearing loss

Images: jaxmice.jax.org
Metal + Noise Mouse Model

- Mouse strain
- Dose of toxicants
- Groups
- Timeline
- Verifying exposures and outcomes
A Mouse Strain with Good Hearing

- No Cdh23<sup>ahl</sup> (accelerated hearing loss gene)
- Low to no rate of ear infection
- No Pde6b<sup>rd1</sup> unlike CBA/J (retinal degeneration)

CBA/CaJ
Toxicants (Chemical and Physical)

- Lead
  - 3 mM in drinking water
  - ~0.00007 mM EPA Action Limit; MCLG=0

- Cadmium
  - 300 μM in drinking water
  - ~0.04 μM EPA MCLG

- Noise
  - 2-hour broadband noise exposure
    - 105 dB (2-20 kHz)
  - ~Chainsaw or Power mower
Exposures Groups

8 Groups

- **Control** (Deionized water) + Sham Noise
- **Noise** (Deionized water)
- **Pb** + Sham Noise
- **Pb** + Noise
- **Cd** + Sham Noise
- **Cd** + Noise
- **Cd** + **Pb** + Sham Noise
- **Cd** + **Pb** + Noise
Timeline

Week 12
Tissue Harvest

Week 11
Final ABR

Week 7-10
Continue Drinking Water Treatment

Week 6
Noise or Sham

Weeks 1-6
Drinking Water Treatment
-Cd
-Pb
-DI water

Weeks 1-6
Drinking Water Treatment

Baseline ABR

AGE: 5 wks

11 wks

16 wks

17 wks
Verification of Exposures

- Blood collection to validate exposures from bottles
- Noise meters measure noise levels in booth before, during and following exposures
- Noise spectrum of mouse housing location
- Acid washing of water bottles
- Metal levels of food and bedding
- Histology of liver and kidney to show systemic health
RESULTS

- Blood Levels (Pb and Cd)
- Auditory brainstem response
- Outer hair cell counts
Blood Pb Levels

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pb</th>
<th>Pb+Noise</th>
<th>Cd+Pb (Pb)</th>
<th>Cd+Pb+Noise (Pb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg Pb/dL whole Blood</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

No significant differences
p-value 0.486

Detection limit
0.5 μg/dL
Blood Cd Levels

* * p-value < 0.0001

Detection limit 0.25 μg/L
ABR
### Outer Hair Cells: Basal Region

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pb</th>
<th>105 dB</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1" alt="Control" /></td>
<td><img src="image2" alt="Pb" /></td>
<td><img src="image3" alt="105 dB" /></td>
<td><img src="image4" alt="Cd" /></td>
</tr>
</tbody>
</table>

- Control
- Pb
- 105 dB
- Cd
Conclusions

- Establishment of an adult mouse model
  - Relevant high levels of blood lead and cadmium
  - Occupational noise exposure levels
- Lack of significant threshold shifts due to metals alone
- Noise was confirmed as detrimental to hearing function
- Potentiation due to metal + noise or metal + metal was not seen in this young adult mouse model
Future Directions

- Less robust mouse strains
- Alternative age and timeline options
- Our study did not address the effects on complex auditory tasks.
  - People often complain that they hear well in quiet environments, not in noisy and loud ones
- Many future mixtures of multiple agents lie ahead!

Images: flickr.com/photos/74418101@N02
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