The Expression of IL-6 in a Rat Model of Infantile Spasms

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Infantile Spasms

- Seizure disorder
- Age of onset is 3 and 12 months
- 2,500 children diagnosed
- Difficult to diagnose
- Extremely detrimental
- Major characteristics
  - Age-specific spastic seizures
  - Hypsarrhythmia
  - Mental retardation

(Dulac, 2001)
Infantile Spasms and Neuroinflammation

- Inflammation of the nervous tissue in the brain (Patel et al., 2013)
- Seizures cause trauma to the brain, releasing inflammatory molecules (Verrotti et al., 2007)
- Inflammatory molecules try to repair the damage causing neuroinflammation (Verrotti et al., 2007)
Inflammatory Molecules

- Inflammatory molecules are released when cells in the body are damaged and in need of repairing (Dupis et al., 2014)
- Cytokines
  - Interferons
  - Interleukins
  - Growth factors
- Secreted by immune system (Vezzani et al., 2015)
- Anti-inflammatory molecules help to suppress inflammation while pro-inflammatory molecules aid in stimulating inflammation
IL-6

- Pro-inflammatory cytokine
- It is secreted by the immune system under trauma, leading to further inflammation of the damaged tissue
- Trauma from seizures leads to the body releases inflammatory molecules
- Either increase or decrease inflammation

(Vezzani et al., 2015)
Research Objective

- Examine the role of IL-6 in the development of Infantile Spasms
- Does prenatal stress affect seizure susceptibility and inflammatory protein expression aftermath?
- What affect do NMDA-induced spasms have on the expression of IL-6?
Outline of Methods

① Betamethasone

② Immunohistochemistry
   a) Primary Antibody
   b) Secondary Antibody
   c) AB Complex
   d) Diaminobenzadine

③ Density Analysis
Betamethasone

- Betamethasone (0.4mg/kg x2) is a drug administered to the rat mothers whilst pregnant at G15 and then sacrificed on gestational day 15
- A way to mimic prenatal stress to see its affect on the mice after they are born and its role in seizure susceptibility to induced Infantile Spasms
- Elevates maternal corticosteroid levels
- Betamethasone vs. control

(Yum et al., 2011)
Outline of Methods

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   a) Primary Antibody
   b) Secondary Antibody
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③ Density Analysis
Immunohistochemistry

- Staining technique used to amplify certain proteins to make easier to visualize cellular components
- Targets antigens by making antibodies bind to a specific antigen
- Makes it possible to block out background in order to make the foreground more prominent

(Wright, 2011)
Primary Antibody
Secondary Antibody
AB Complex

Diagram showing the interaction between Avidin, Biotin, Antibody-Biotin conjugation, and HRP-Biotin conjugation.
Diaminobenzidzidine (DAB)
Outline of Methods

① Betamethasone
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③ Density Analysis
Density Analysis

- Subtract the foreground intensity from the background
- Sent from microscope to computer to be analyzed
- Five samples from both foreground and background
- Higher the number the greater the increase in the difference between the foreground and the background

![ImageJ software interface](image.png)
Results: Density Analysis

- Comparison between density analysis performed on several slices of the mice brains administered primary antibody IL-6 versus the control sample.
- The numbers are compared to the controls which are typically around 16 identify if more or less proteins are being accounted for.
Relative IL-6 staining density (AU)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>% of total variation</th>
<th>P value</th>
<th>P value summary</th>
<th>Significant?</th>
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<tr>
<td>Interaction</td>
<td>17.88</td>
<td>0.0947</td>
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<tr>
<td>Postnatal NMDA</td>
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<td>0.0488</td>
<td>*</td>
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<td>Prenatal treatment</td>
<td>9.337</td>
<td>0.2103</td>
<td>ns</td>
<td>No</td>
</tr>
</tbody>
</table>
Results; Beta vs. Saline

- Controls had a foreground to background density number of roughly 16
- Above 16 would imply there is greater background to foreground staining, therefore there are more positive proteins being counted
- There was a lower number and lighter staining in the beta animals than the saline animals
  - 25% decrease in color density which means there was a decrease in the IL-6 presence in the beta animals
- NMDA significantly affects expression however prenatal priming does not
Results; Beta vs. NMDA

- After an insult, such as NMDA-induced spasms, there is an overcompensation and increased expression.
- Increased presence may also contribute to differences in seizure threshold in beta animals, which are lower.
- NMDA significantly affects expression however prenatal priming does not.
Conclusion

- Prenatal stress (ie.beta) affects the development of the immune system during infancy → affecting seizure susceptibility
- Beta+NMDA has an increase in IL-6 after spasms which is not present in saline
- Priming alone does not affect baseline expression of IL-6 in the superior cerebellar peduncle
- Although beta priming does not immediately affect IL-6 expression or regulation, it does change the regulatory pathways associated with controlling its expression
- This knowledge will contribute to a better understanding of the pathology of infantile spasms leading to development of better, more effective treatments in the future
Future Research

- In the future, we would like to continue this investigation to further confirm our results in order to see how IL-6 and other proinflammatory molecules can be used as possible treatments for Infantile Spasms.
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Bibliography