Interactions of Sex and Neuroimmune Function and the Risk of Neurodevelopmental Disorders

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Your Immune System Affects Your Behavior.

For example, you get sick.

“Adaptive” Immune Function

Pathological Immune Function

Immune dysregulation has been associated with:
- Autism
- Schizophrenia
- Depression
- Cerebral Palsy
- Delirium

Sickness Behaviors
- Reduced Appetite
- Increased Sleep/Decreased Activity
- Reduced Social Interactions
- Altered Cognitive Function

Behavioral Symptoms of Neuropsychiatric Disorders
- Chronic metabolic disturbances
- Chronic sleep disturbances, fatigue, lack of energy
- Altered social interactions / increased withdrawal and depression
- Decreased cognitive function (learning disabilities, dementia, or delirium)
Microglia are the immune cells in the brain.

- Microglia respond to insult, injury, or infection with the goal of restoring homeostasis in the brain.

- Neurons are very sensitive to the pro- and anti-inflammatory molecules produced by microglia.

- Thus microglia and the immune molecules they produce can significantly affect neural function and behavior.

Red = microglia
Green = neurons
Early-Life Programming of Later-Life Brain Function and Behavior.

Events that occur early in development can produce profound deviations in normal development with significant effects on physiology and behavior later in life.

Immune Function
Does activation of the immune system during development produce long-term changes in immune function that impact behavior?
Immune Activation early in Development is Linked to Neurodevelopmental Disorders.

- Perinatal exposure to infection and subsequent inflammatory responses have been implicated in the etiology of autism, cerebral palsy, and schizophrenia. (Meyer et al., 2011; Brown et al., 2010; Libbey et al., 2005; Dammann et al., 2000).

- Individuals with autism, cerebral palsy, and schizophrenia have altered immune function = increased antibody production, altered T, B, and NK cell responses, altered cytokine production, and increased incidence of allergies. (Pardo et al., 2005; Atladottir et al., 2009; Keil et al., 2010; Van de Water 2005).

- In addition, early life immune activation or dysregulation is associated with generalized pervasive developmental disorders [learning disabilities, emotional dysregulation] (Fombonne E, 2009).

- Many of the epidemiological data indicate that these neurodevelopmental disorders likely result from “two-hits”. Thus the “two-hit hypothesis of neurodevelopmental disorders” has been postulated.
How does early-life immune **activation** impact later-life immune function, brain function, and behavior?

Microglia are important for:
- Neurogenesis
- Synaptogenesis
- Cell migration
- Synaptic pruning
- Programmed cell death

**Perinatal Challenge**
- Infection
- Drugs of abuse
- Alcohol
- Stress

**Adult Immune Challenge** (Infection)
- Cytokines
- Chemokines

**Amoeboid / Activated**
- IL-1β
- TNF α
- IL-6

**Ramified / Quiescent**

**Infiltration of Mononuclear Cells from the Periphery**

**“Sensitive Period”** for long-term changes in neuroimmune function and behavior.

(Bilbo and Schwarz, FIBN, 2009)
Early-life immune activation results in exaggerated neuroimmune function in adulthood.

(Bilbo and Schwarz, FIBN, 2009)
Sex differences in the frequency of Neurodevelopmental Disorders

<table>
<thead>
<tr>
<th>Developmental Disorders</th>
<th>M : F</th>
</tr>
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<tbody>
<tr>
<td>Autism</td>
<td>2.5 : 1</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>2.5 : 1</td>
</tr>
<tr>
<td>Dyslexia</td>
<td>3 : 1</td>
</tr>
<tr>
<td>Mental Retardation</td>
<td>1.6 : 1</td>
</tr>
<tr>
<td>Stuttering</td>
<td>3 : 1</td>
</tr>
<tr>
<td>ADHD</td>
<td>10 : 1</td>
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</tbody>
</table>

Disorders that are either diagnosed or have their origins in development.

Many considered disorders of “wiring” in the brain.
Does sex influence microglial number in the brain?
Males Females

Postnatal Day 4

Cortex
CA1 CA3
DG
Amygdala

Hypothesis

Males may be more vulnerable to the developmental consequences of neonatal immune activation because they have more microglia in the developing brain that produce a significantly greater immune response to an infection.
Examine the neuroimmune and peripheral immune response to a neonatal infection in both males and females.

P0 = birth

E.coli (1 X 10^6 C.F.U) infection

or

Sterile Phosphate-buffered Saline

8 hours later

Collect tissue/serum

24 hours later

Collect tissue/serum

P4

P5

Analysis:

Real-time PCR of inflammatory gene expression in brain and periphery.
Contrary to predictions, we found no significant difference in the neuroimmune response to a neonatal infection.

*p < 0.05 Main Effect of Infection
There was a significant interaction of sex and neonatal infection on the immune response measured in the periphery - **Spleen**

Males exhibit a significantly **greater peripheral** immune response to a neonatal infection than females.

* p < 0.05 significant effect relative to same sex control

** p < 0.05 relative to female infected pups.
Examine the impact of neonatal infection on neuroimmune and peripheral immune function in juvenile rats.

- **E.coli** (1 X 10^6 C.F.U) infection
  - or
  - Sterile Phosphate-buffered Saline

“First Hit”

- 4 hours later Collect tissue/serum
- Treat with saline
  - or
  - lipopolysaccharide (LPS, 25 μg/kg)

“Second Hit”

Why Postnatal day 24?
Neonatal infection produces long-term changes in gene expression within the **hippocampus** of juveniles.

- Neonatal infection results in elevated levels of IL-1β expression **three weeks** post infection.
- Neonatal infection results in decreased levels of BDNF expression nearly three weeks post infection.
- A “second hit” produces exaggerated IL-1β expression in both neonatally infected males and females, and inhibits BDNF expression overall.
Neonatal infection produces long-term changes in gene expression within the peripheral immune system of juveniles.

**Spleen IL-1β**

**Spleen IL-6**

**Spleen TNFα**

IL-6

Interaction of Sex X Neonatal Trmt X LPS, * p < 0.05
Neonatal infection produces long-term changes in the number of circulating WBCs in the peripheral immune system of juvenile males.

- **Basophils** are important in allergic responses – they produce histamine.
- **Eosinophils** are important in regulating inflammation in response to allergies / asthma.
Conclusions from our Data

• **Neonatal infection produces**
  - a more robust immune response *in neonatal males*.
  - long-term changes in the expression of cytokines (↑IL-1β) and neurotrophic factors (↓BDNF) *particularly in males*.
  - long-term changes in the expression of peripheral cytokine expression (↓IL-6 in spleen) *particularly in males*.
  - Long-term increases in the circulation of WBCs involved in allergy / asthma, *particularly in males*.

Males may be more vulnerable to immune activation early in development, which significantly impacts immune function, the risk of allergic responses, and cognitive / behavioral deficits as juveniles.
Researchers are investigating the potential benefits of minocycline on developmental disorders.

A pilot open-label trial of minocycline in patients with autism and regressive features

Carlos A Pardo, Ashura Buckley, Audrey Thurm, Li-Ching Lee, Arun Azhagiri, David M Neville and Susan E Swedo

[Graphs showing serum CXCL8 (IL-8) levels pre and post-treatment with p-value of 0.047.]
Researchers are investigating the potential benefits of minocycline on developmental disorders.

A Randomized Double-Blind, Placebo-Controlled Trial of Minocycline in Children and Adolescents with Fragile X Syndrome

Mary Jacena S. Leigh, MD,† Danh V. Nguyen, PhD,‡ Yi Mu, MS,‡ Tri I. Winarni, MD,§ Andrea Schneider, PhD,† Tasleem Chechi, BS,°† Jonathan Polussa, BS,°† Paul Doucet, BA,† Flora Tassone, PhD,†∥ Susan M. Rivera, PhD,∥∥ David Hessl, PhD,∥∥∥ Randi J. Hagerman, MD∥∥∥

The authors found an improvement on the Clinical Global Improvement Score and a decrease in anxiety and mood-related behaviors following three months of treatment with minocycline in children with Fragile X.
Despite the growing interest in microglia and immune cells as a target for developmental disorders...

- There are no papers examining the effects of minocycline on microglia in juvenile rodents.

- Very little (only two papers) examining the effectiveness of minocycline on female rodents.
Additional Questions and Future Directions

• **Future Goals:** Fully characterize the immune system throughout development in both males and females, which will likely provide insight into vulnerabilities to neurodevelopmental disorders as well as potential therapeutic targets.

• Understand the function of peripheral immune cells as they relate to neurodevelopment and the risk of behavioral, mood and learning disorders, particularly in males.

“Many neuropsychiatric disorders begin early in life and thus are chronic disease of the young that have become the largest source of years lived with disability”.

(Insel and Landis, *Neuron*, 2013)
Thank You

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