Sex-specific Effects of Unpredictable Variable Prenatal Stress: Implications for Mammalian Developmental Programming During Spaceflight

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INTRODUCTION

During adaptation to the microgravity environment, adult mammals experience stress mediated by the Hypothalamic-Pituitary-Adrenal axis. In our previous studies of pregnant rats exposed to 2-g hypergravity via centrifugation, we reported decreased corticosterone and increased body mass and leptin in adult male, but not female, offspring. In this study, we utilized Unpredictable Variable Prenatal Stress to simulate the stressors of spaceflight by exposing dams to different stressors. Stress response modulation occurs via both positive and negative feedback in the hypothalamus, anterior pituitary gland, and adrenal cortex resulting in the differential release of corticosterone (CORT), a murine analog to human cortisol.

In this study, we exposed non-manipulated, Gestational Day 0 (GD) dams to Unpredictable Variable Prenatal Stress (UVPS), raising the resultant offspring to Postnatal Day 90 (P90) followed by sacrifice and processing of tissues for RNA purification, cDNA synthesis, and RT-qPCR. In addition to the primary HPA genes resulting in CORT release, the following genes were additionally analyzed via RT-qPCR in the same tissues:

**Hypothalamus**
- Corticotropin-Releasing Hormone (CRH)
- Arginine Vasopressin
- Glucocorticoid Receptor (GR)
- Pro-opiomelanocortin (POMC)

**Anterior Pituitary**
- Brain-Derived Neurotrophic Factor
- CRH Receptor
- Vasopressin Receptor 1B

**Adrenal Glands**
- Tyrosine Hydroxylase

**METHODS**

1. Prenatal Stress
2. Maturation to P90
3. Sacrifice and Tissue Processing
4. RNA Purification and cDNA Synthesis
5. RT-qPCR

**RESULTS**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Gene of Interest</th>
<th>N (per group)</th>
<th>Male NS</th>
<th>Female NS</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>Corticotropin-Releasing Hormone</td>
<td>7</td>
<td>4.0E-6</td>
<td>7.1E-6</td>
<td>2.0E-4</td>
</tr>
<tr>
<td>Anterior Pituitary</td>
<td>Arginine Vasopressin</td>
<td>8</td>
<td>Expression below detectable threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucocorticoid Receptor</td>
<td>8</td>
<td>2.0E-2</td>
<td>1.4E-3</td>
<td>6.0E-2</td>
</tr>
<tr>
<td></td>
<td>Pro-opiomelanocortin</td>
<td>8</td>
<td>2.0E-2</td>
<td>1.4E-3</td>
<td>6.0E-2</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor</td>
<td>CRH Receptor</td>
<td>8</td>
<td>1.4E-2</td>
<td>1.7E-2</td>
<td>0.11</td>
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<tr>
<td></td>
<td>Vasopressin Receptor 1B</td>
<td>8</td>
<td>3.0E-5</td>
<td>3.0E-5</td>
<td>0.01</td>
</tr>
<tr>
<td>Adrenal Glands</td>
<td>Tyrosine Hydroxylase</td>
<td>5</td>
<td>1.5E-1</td>
<td>1.7E-1</td>
<td>1.6E-2</td>
</tr>
</tbody>
</table>

**CONCLUSION**

1. Of the genes studied, Brain-Derived Neurotrophic Factor and Arginine Vasopressin in the Hypothalamus did not show consistent ACT values relative to the GAPDH housekeeping gene, and no there are no conclusive data to determine their role in stress-axis response.

2. No significant differences in gene expression were noted in CRH (Hypothalamus), POMC (Hypothalamus), Vasopressin Receptor 1B (Pituitary), and Tyrosine Hydroxylase (Adrenal Glands). These genes did not show a lasting differential level of expression in adult prenatally stressed rats.

3. A trend (p = 0.07) was noted in the levels of Glucocorticoid Receptor (GR) expression between sexes with no significant treatment effect. Average GR expression was higher in male rats than in female rats, suggesting that a basal difference in sensitivity to glucocorticoids may be present in adult animals independent of stress exposure. However, further study is needed to confirm this trend.

4. A trend (p = 0.11) was noted in CRH Receptor (CRHR1) expression. A Tukey-Kramer post-hoc analysis revealed a significant effect between both sex and treatment (p = 0.04), suggesting that sex-specific response to stress may become present with a larger sample size. However, the non-significant main effect requires further experimentation to confirm this trend.

**STUDY IMPROVEMENTS**

1. Failure in two of the eight genes relative to the housekeeping gene suggests alternate primers may be needed to show consistent expression. Additionally, specific regions of the organs studied may show differential expression in genes and may require regional dissection prior to analysis.

2. The non-significant expression levels in the other genes studied may be the result of whole-organ homogenization. Analysis of expression in specific regions (such as the anterior lobe only in the case of corticotropin-releasing hormone) may offer greater specificity in terms of localized expression during stress response.

**ACKNOWLEDGEMENTS**

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