Differences in habenula kisspeptin expression and its effects on stress coping styles in zebrafish, *Danio rerio*

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**Background**

Stress Coping Styles

<table>
<thead>
<tr>
<th>Proactive</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive</td>
<td>Assertive</td>
</tr>
<tr>
<td>Avoidance</td>
<td>Avoidance</td>
</tr>
</tbody>
</table>

**Habenulo-Raphe Connection**

- The habenula has afferent and efferent connections to major forebrain networks (e.g., limbic system, basal ganglia) and the monoaminergic hindbrain (e.g., raphe)
- These connections are also seen in many other vertebrates

**Hypothesis**

Proactive zebrafish will be more sensitive to kisspeptin and this plays a role in the increased 5-HT expression.

**Materials and Methods**

- 12 proactive and 12 reactive (36 ug) zebrafish were sacrificed
- Half male/half female
- Mean +/- standard error

**Results**

<table>
<thead>
<tr>
<th>Permutation</th>
<th>Hybridization Temp</th>
<th>Blocking Buffer</th>
<th>Antibody</th>
<th>Fluorescent</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>65°C</td>
<td>1X LMM</td>
<td>HRP</td>
<td>0.5X</td>
<td>Low</td>
</tr>
<tr>
<td>II</td>
<td>67°C</td>
<td>0.5X HRP</td>
<td>Poly-HRP</td>
<td>0.25X</td>
<td>High</td>
</tr>
<tr>
<td>III</td>
<td>67°C</td>
<td>0.5X HRP</td>
<td>Fluorescent</td>
<td>0.25X</td>
<td>Moderate</td>
</tr>
</tbody>
</table>


**Conclusion and Discussion**

- Despite trying to optimize the in situ hybridization reaction parameters by running ten different permutations, we have yet to identify a parameter that demonstrates our probe is specifically binding to kiss1ra mRNA
- Further investigation will be required to determine the specific issue
- Given that we see signal on both antisense and sense it is possible that the issue lies in the probes specificity for binding to only kiss1ra
- Alternate primers for kiss1ra mRNA will be reviewed

**References**