Histological Analysis of the Neurodevelopmental Effects of Corticosterone in Northern Leopard Frog (Lithobates pipiens) Tadpoles

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INTRODUCTION:
Chlorpyrifos (CPF), an organophosphorus pesticide, is found throughout the environment and affects many organisms, including humans.¹,² Levels are typically low and are considered safe by the government. However, trace levels of CPF alter neurodevelopment of Northern Leopard Frogs (Lithobates pipiens), a common vertebrate model.³,⁴ These trace amounts of pesticide resulted in tadpoles with enlarged brain morphology and increased corticosterone, a metabolic and stress hormone.³,⁴

We hypothesized that the increased corticosterone contributed to the pesticide-induced changes in neurodevelopment. To test this, we exposed tadpoles to either corticosterone or a vehicle for one week and measured tadpole corticosterone and brain morphology using a morphometric method. Using the morphometric method, we found that the diencephalon was wider in animals treated with CORT.

The morphometric approach showed an increase in diencephalon width in the brains exposed to corticosterone compared to those exposed to the vehicle.

HYPOTHESIS & PREDICTIONS:
Here, we tested the hypothesis that the change in diencephalon width after exposure to CORT found using morphometric methods would be apparent using histological methods.

We predicted that the dimensions, measured using histological methods, of the diencephalon, and its component parts, the thalamus and hypotalamus, would be different in CORT exposed subjects compared to vehicle-exposed controls.

METHODS:

1. Exposure to Treatments:
- Northern leopard frog tadpoles (Gosner stages 37–40)
- 11 tadpoles per treatment in individual tanks
- 1 week exposure

2. Cryosection and Staining:
Brains were fixed in 4% paraformaldehyde and sectioned using a cryostat and mounted onto slides with a coverslip and paramount. Each slide then went through a staining process with cresyl violet.

3. Histological Image Analysis:
I created a brain atlas to determine the specific regions of interest. ImageJ was used to take measurements of four specific regions of the brain: diencephalon width, diencephalon height, area of thalamic nuclei, and area of hypothalamus. Yellow highlight and arrows are indicative of the specific regions that were measured.

Brain atlas showing regions of interest: TN: thalamic nuclei; III: third ventricle; HYP: hypothalamus; OT: optic tectum; I: infundibulum; AS: aque ductus sylvii; OV: optic ventricle.

REFERENCES:

KEY RESULTS:

P-value* 0.92
Covariate Brain mass

P-value* 0.31
Covariate Brain mass

Other Results:

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Mean (Vehicle)</th>
<th>Mean (CORT)</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Area Thalamic Nuclei (mm²)</td>
<td>1607.14 ± 0.001</td>
<td>1748.09 ± 0.001</td>
<td>0.63</td>
</tr>
<tr>
<td>Average Area Thalamic Nuclei (mm²)</td>
<td>614.02 ± 0.001</td>
<td>559.37 ± 0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Total Area Hypothalamus (mm²)</td>
<td>980.88 ± 0.001</td>
<td>925.35 ± 0.001</td>
<td>0.64</td>
</tr>
<tr>
<td>Average Area Hypothalamus (mm²)</td>
<td>233.11 ± 0.001</td>
<td>226.12 ± 0.001</td>
<td>0.69</td>
</tr>
<tr>
<td>Brain mass (g)</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.001</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*1-way ANOVA, with applicable covariates, was performed using SPSS.

CONCLUSIONS:
Using histological methods, we sectioned the brain and measured the size of the diencephalon and several of its subregions. We found no effect of corticosterone on any measurements of diencephalon size. Thus, the morphometric approach is more sensitive to detecting corticosterone-induced brain changes than the histological approach, perhaps due to the extra processing steps involved in histology that potentially distort the tissue.

TAKE HOME MESSAGE:
It is important to develop sensitive methods to detect changes in neuroanatomy induced by environmental stressors such as pesticides or other water contaminants. Our study indicates that the morphometric approach is more sensitive to anatomical changes than the histological method.

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