Measuring the self-correction of brain morphology in pre-metamorphic *Xenopus laevis* tadpoles by examining markers of brain tissue differentiation and cerebrospinal fluid flow

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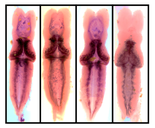
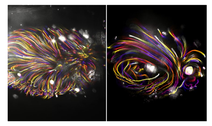


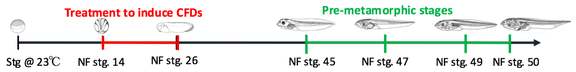


Introduction:

Craniofacial deformities are common developmental defects across all vertebrate species, which can be caused by genetic factors, environmental factors, or a combination of both. Typically in humans, these birth defects (e.g., cleft palate, fetal alcohol syndrome and microcephaly) can only be treated with an expensive and invasive surgery. These exterior craniofacial abnormalities are often correlated with brain abnormalities as well, which can lead to behavioral and cognitive delays. Thus, there is a critical need to study not just the causes of craniofacial defects in vertebrate model systems, but also potential resolutions and treatments for craniofacial defects. It was observed by our collaborators in the Levin Lab at Tufts University that *Xenopus laevis* tadpoles with malformed craniofacial features are able to normalize over time. The McLaughlin lab took this information and discovered that these tadpoles can remodel malformed cartilage and brain tissue prior to and independent from metamorphosis. The McLaughlin lab is now working to elucidate the underlying mechanisms that direct the self-correction of malformed tissues in the heads of *Xenopus laevis* tadpoles, with the ultimate goal of supporting the discovery of novel treatments for correcting human craniofacial birth defects. To help this objective, my project focused on determining whether brain tissue in pre-metamorphic tadpoles with craniofacial defects is properly differentiated prior to and after the life stages where malformed craniofacial tissues are known to correct. The second part of my project was to examine the cerebrospinal fluid flow in the brains to determine whether or not there was a blockage of flow in the malformed brains, and whether this is corrected over time as well.

Immunohistochemistry Workflow:

1. Expose neurula stage embryos to one of the following chemical treatments to generate tadpoles with craniofacial defects: ICI 118,551, thioridazine HCl, or ethanol.



2. MEMFA-fix tadpoles at NF stages 45, 47, 49 and 50.
3. Bleach tadpoles and dissect out the brains.
4. Perform immunohistochemistry procedure targeting the Pax7 protein to discern cell differentiation in the brains.
5. Score the brains as having normal or abnormal protein expression patterning and perform Chi-Square Analysis to find significant experimental values in the data.

Results:

Is Pax7 protein expression abnormal in the brain tissue of tadpoles with brain defects? If so, does it normalize prior to metamorphosis?

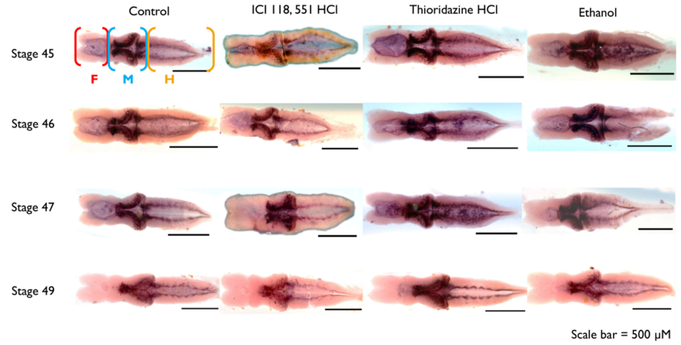


Figure 1. Images of control, ICI 118,551, thioridazine HCl and ethanol tadpole brains at NF stages 45, 46, 47 and 49 showing the Pax7 protein expression pattern. Dark purple pigment = Pax 7. All three experimental groups were treated only during neurulation.

Yes, treatment group tadpoles show abnormal Pax7 protein expression patterns at stage 45 and by stage 49, the Pax7 protein expression pattern appears normalized and indistinguishable from the controls.

Results:

Phenotype scoring reveals that abnormal Pax7 expression significantly improves by stage 49 in post-ICI 118,551 and post-ethanol brains.

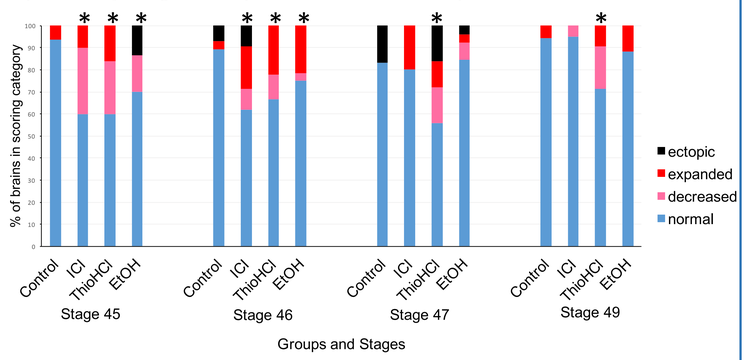
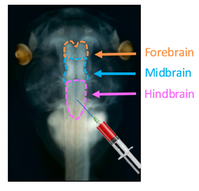


Figure 2. Percentage of protein expression in the brains control, ICI 118,551, thioridazine HCl, and ethanol groups at NF stage 45, 46, 47, and 49. Blue = brains with normal expression pattern, pink = decreased expression, red = expanded expression, and black = ectopic expression. A Chi-Square goodness of fit test was performed, * = p<0.05. N = 3 trials, n = 10-32 brains per group per stage.

Cerebrospinal Fluid Imaging Workflow:

1. Obtain albino eggs from albino adult female frogs and fertilize using the gonads of an adult albino male.
2. Expose neurula stage embryos to one of the following chemical treatments to generate tadpoles with craniofacial defects: ICI 118,551, thioridazine HCl, or ethanol.
3. Inject fluorescent microbead solution into the hindbrain of tadpoles at stages 45-50.

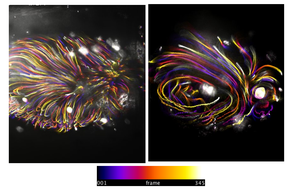


4. Image and record videos of the microbead flow within the brain ventricles using a Zeiss microscope.
5. Visualize the trajectories and measure the velocity of the microbeads in the brains of the different treatment groups.

Results:

Do abnormal brain morphologies correspond with abnormal cerebrospinal fluid flow? If so, is abnormal cerebrospinal fluid flow be corrected along with abnormal brain morphologies?

Figure 3. Microbead trajectories within the hindbrain of a control brain (left) and thioridazine HCl brain (right) at stage 45. The colored lines mark the trajectories of individual beads throughout the 5 second video, and the color scale below indicates the number of frames each bead was found to be moving.



Yes, the microbead trajectories in the treatment group brains are elongated at stage 45 relative to controls, indicating slower cerebrospinal fluid flow. However, by stage 49 the cerebrospinal fluid flow of treatment groups normalize; the velocities of the beads in the treated brains are not statistically significantly different from those in control brains by stage 49.

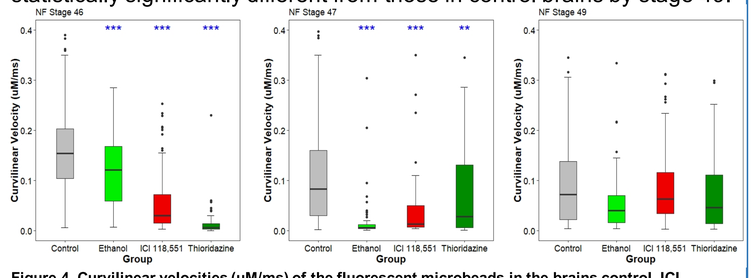


Figure 4. Curvilinear velocities (uM/ms) of the fluorescent microbeads in the brains control, ICI 118,551, thioridazine HCl, and ethanol groups at NF stages 46, 47 and 49 (from left to right). A Chi-Square goodness of fit test was performed: *** = p<0.0001, ** = p<0.001, * = p<0.05. N = 3 trials, n= approximately 15 tadpoles per group per stage.

Conclusions:

Malformed, or abnormal brains in *Xenopus* laevis pre-metamorphic tadpoles have shown to self-correct over time in three ways:

- Cell level differentiation, as can be seen in the improvement the Pax7 protein expression pattern from stage 45 to 49
- Overall structure and morphology of the brain
- Functionality of the brain with regards to cerebrospinal fluid flow

Acknowledgements:

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The Paul G. Allen Frontiers Group