Altered Left/Right Brain Asymmetry Disrupts Normal Behavior of *Ciona* Larvae

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1. Tunicates Are Model Organisms for Neurobiology

Tunicates are chordates that are closely related to vertebrates. Larvae of the tunicate *Ciona intestinalis* have only 177 neurons, providing a simple model for studying the connections between brain and behavior.[1] *Ciona* larvae swim up in response to dimming light, a behavior called gravitaxis.[2] We studied how dechorionation, a common laboratory technique that removes the protective layer (chorion) from the egg, affects this behavior. Resting on the basic biological principle that structure underlies function, we hypothesized that behavior might be altered because dechorionation has been observed to disrupt the normal anatomical left/right asymmetry of the larval brain.

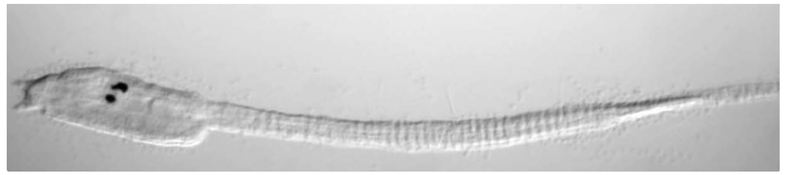

Figure 1. *Ciona* larva.

2. Using Gravitaxis to Study Dechorionated Larvae

We studied the behavior of larvae dechorionated at an early developmental stage, dechorionated at a later stage, and not dechorionated at all (control) using the gravitaxis assay.[2] Scoring of larvae swim directions was done manually. A MATLAB script was used to track larval swimming.

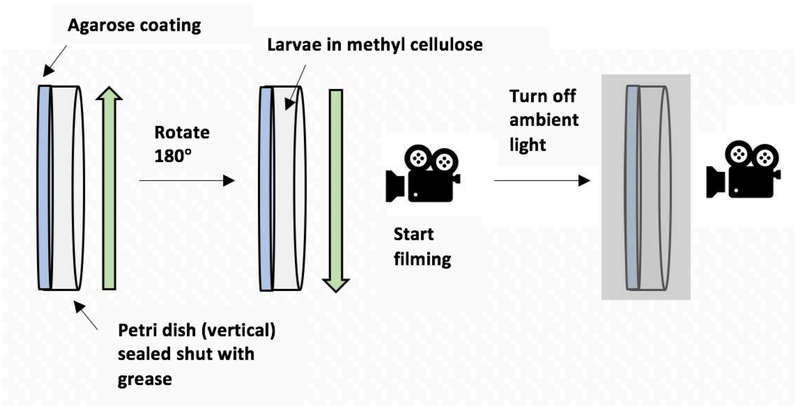

Figure 2. Gravitaxis behavioral assay set-up. Allows larvae to be suspended in solution, able to swim in all directions upon dimming. Only larvae responding to the dim are scored for swimming direction.

3. Dechorionation Disrupts Swimming Patterns

While all controls and late dechorionated larvae responded to dimming, only 50% of early dechorionated larvae did. Early dechorionated larvae also displayed impaired gravitaxis abilities, compared to their controls. Late dechorionated larvae appear to be able to gravitax. Both early and late dechorionated larvae swam at slower speeds than their control counterparts.

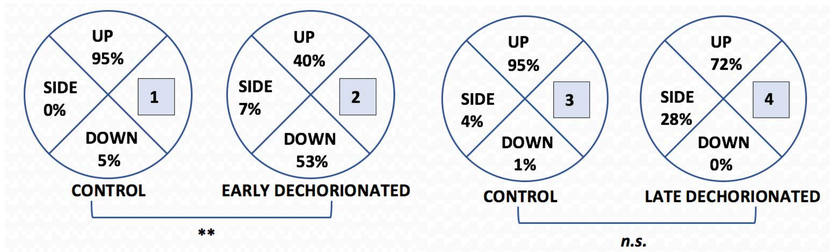

Figure 3. Percentages of larvae swimming in each direction after the dim in gravitaxis assay (side = sideways). Controls (1) and early dechorionated (2) are significantly different (** = p<0.01). Controls (3) and late dechorionated (4) are not significantly different (n.s.); they both tend to swim up.

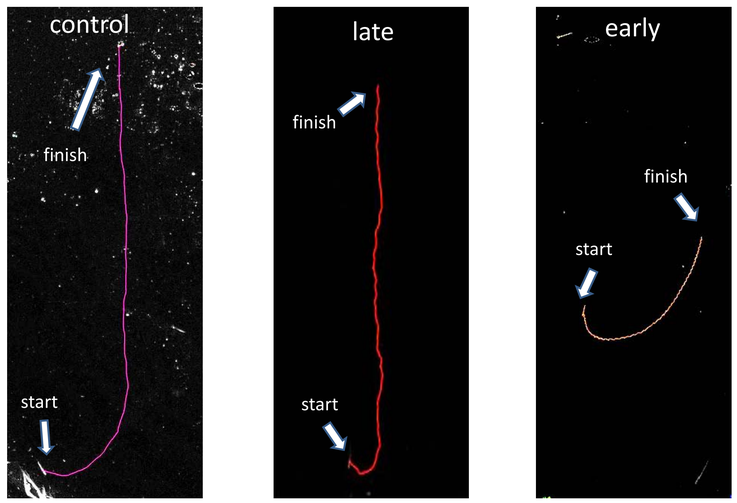

Figure 4. Swimming paths of control larvae (not dechorionated), late dechorionated larvae, and early dechorionated larvae. Controls and late dechorionated show a stronger upward swimming pattern than early dechorionated larvae.

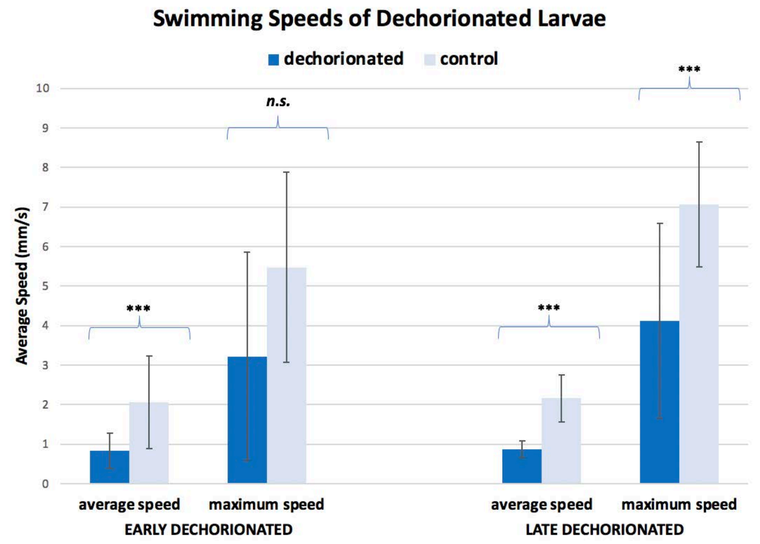

Figure 5. Early dechorionated larvae display significantly decreased average swimming speeds compared to controls (*** = p<0.001). Late dechorionated larvae also swim significantly slower than controls.

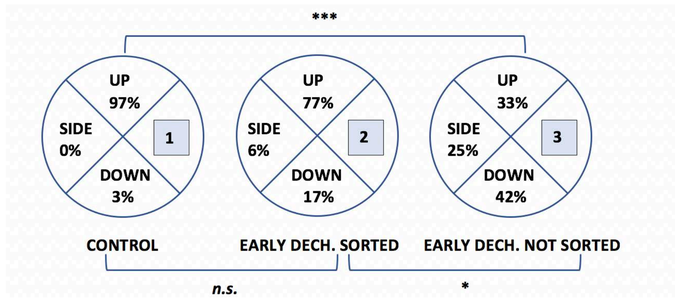

Figure 6. Early dechorionated larvae sorted for dimming response (2) appeared to gravitax. Their unsorted siblings (3) could not.

4. Dechorionation Is Still a Powerful Technique

Researchers should proceed cautiously when drawing conclusions from experiments that require dechorionation, especially at early stages, because of the effects on behavior. Sorting larvae can allow researchers to accurately attribute behavioral results to their intended manipulation. This sorting technique also provides a powerful alternative to tedious microinjections of gene constructs, making possible future studies of *Ciona* neurobiology.

Contact Information
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References

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2. Bostwick, M., Smith, E. L., Borba, C., Newman-Smith, E., Guleria, I., Kourakis, M. J., & Smith, W. C. (2020). Antagonistic inhibitory circuits integrate visual and gravitactic behaviors. *Current Biology*, *30*(4), 600-609.

Acknowledgments

This project was made possible by the generosity of supporters to the Bruce H. and Robin G. Tiffney Summer Undergraduate Research Fund.