Proper Blastema Formation Requires Innervation in *Aeolosoma* (Annelida)

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Background Information

Calcium channels are vital to neural signaling, specifically in the release of neurotransmitters from axon terminals. In invertebrates, N-type calcium channels assist in neural signaling. Invertebrate calcium-activated chloride channels affect ion transport across cell membranes and help regulate cell homeostasis as well as neural signaling capacity (Wang et al. 2013). *Aeolosoma* is a freshwater annelid capable of complete regeneration when bisected. We tested whether an N-type Calcium Channel inhibitor and a calcium-activated chloride channel inhibitor would have on cell proliferation and neural regeneration. We found that both inhibitors limited the success of regeneration and had a variety of non-normal effects of neural regeneration.

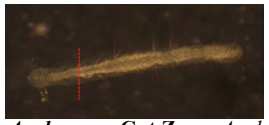

Figure 1: *Aeolosoma* Cut Zone. *Aeolosoma* were cut directly above the midgut at the location indicated by the red line

Results

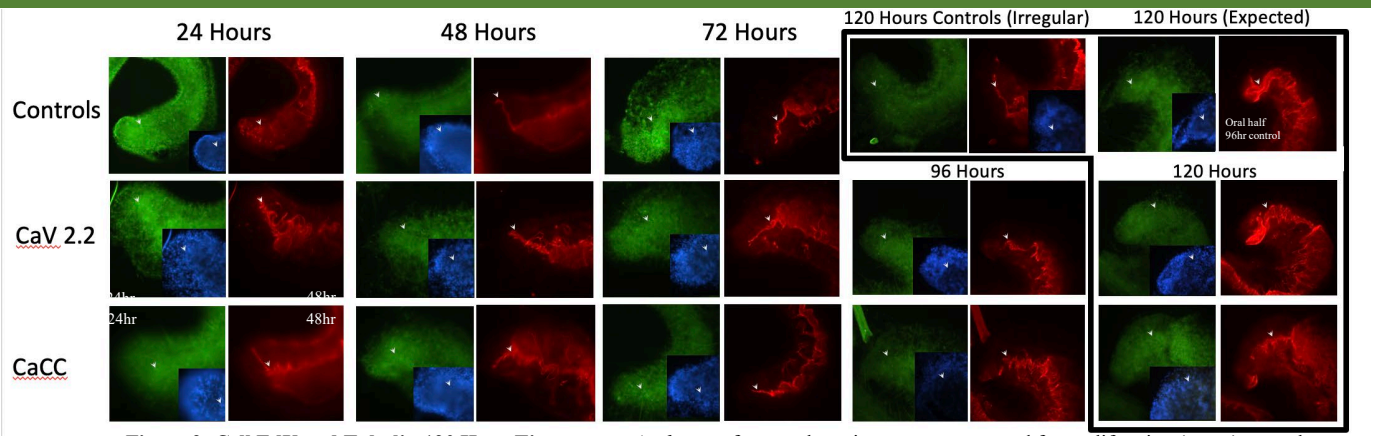

Figure 2: Cell EdU and Tubulin 120 Hour Timecourse. *Aeolosoma* from each environment were tested for proliferation (green), neural cells (red), and nuclei (blue) at each time point. Corresponding cells are indicated by arrows. Focused 96 Hour Control picture were unattainable

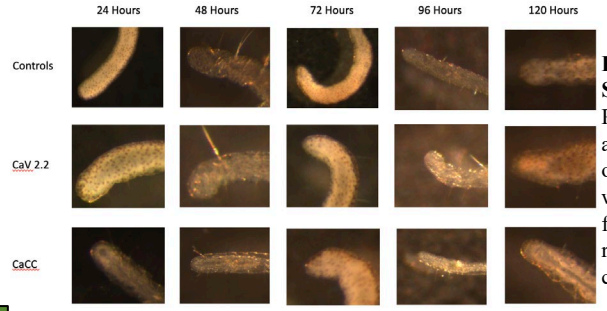

Figure 3: Live Sample Images: Pictures were taken at each time point on live samples without any stain for observable regeneration comparisons

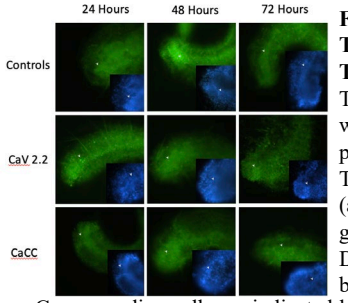

Figure 4: Tunel 72 Hour Timecourse The procedure was repeated partly with Tunel (apoptosis, green) and Dapi (nuclei, blue) staining. Corresponding cells are indicated by arrows. Note the lack of apoptotic cells.

Methods

1.Samples were cut above the midgut on the anterior side (Figure 1). They were then distributed evenly into controls, 0.1 mM Cav2.2, and CaCC inhibitors (Selleckchem).
2.Samples were collected from each environment every 24 hours over the next five days
3.Cell proliferation was observed in each time stamp by labeling cells with the thymine analog 5-ethynyl-20 -deoxyuridine (EdU), and Tubulin staining was used to observe neural regeneration throughout the time course
4.Both EdU and Tubulin stains were applied in the same procedure and successfully stained

Conclusions

1.We successfully combined the EdU and tubulin protocols for the first time.
2.Samples treated with Cav 2.2 and CaCC had difficulty forming blastemas and did not regenerate fully over the usual five-day period.
3.Treated samples showed limited innervation of the blastema and irregular axon formation, suggesting innervation is essential for proper regeneration.
4.Proliferation in treated samples was notably lower than in the controls

References

Wang et al. 2013. Phylogenetic, expression, and functional analyses of anoctamin homologs in *Caenorhabditis elegans*. American Journal of Physiology.