

Repercussions of GlyCl Channel Modulation in *H. glaberrima's* Intestine Regeneration

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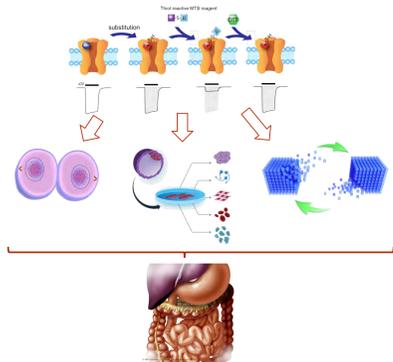
Abstract

Regeneration can be defined as the reactivation of development in later life to restore missing tissue. It is known that the nervous system plays a certain role during this whole process. The voltage gradient in cell membranes serves as instructive signals regulating cell proliferation, differentiation and cell migration, key processes for regeneration. The standing gradients of these voltages along with the ion fluxes among cells serve as regulators for initiating the development of specific organs and the regeneration of whole appendages. In this investigation we aim to determine if the modulation of the GlyCl channel, known for mediating inhibitory and excitatory neurotransmission, has any repercussions in the regenerative ability of the *H. glaberrima's* intestine. The GlyCl channel, when activated, results in a Cl⁻ flux that may cause either a depolarization or a hyperpolarization in the cell. Through the administration of a highly specific drug called Ivermectin we intend to open the GlyCl channel causing a hyperpolarization in the cells of the sea cucumber, leading to a disruption of the action potential and ultimately inhibiting the cell proliferation, among other processes. Through whole mounts we will be able to distinguish innervation of nervous filaments in the mesentery of the sea cucumber and identify if the hyperpolarization of the GlyCl channel inhibits this process. Also it will be possible to measure the SLS's of the blastema and mesentery through Phalloidin staining in 20µm transversal cuts. Preliminary results from the transversal cuts of the intestine confirms the accuracy of the cryostat technique, validating the selection of methodology. However, we plan to explore more profoundly the effect of Ivermectin in the sea cucumber via in-vitro experimentation. A muscle cell extraction from the *H. glaberrima's* radial muscle will be cultured and treated with different conditions to explore if the drug has any effect on the morphology of the cells.

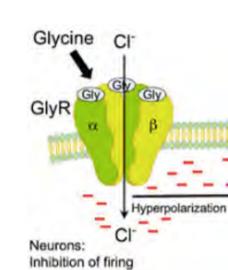
Background

- Regeneration
 - The reactivation of development in later life to restore missing tissue

Role of Nervous System in Regeneration



The GlyCl Channel



Holothuria glaberrima's Intestine Regeneration

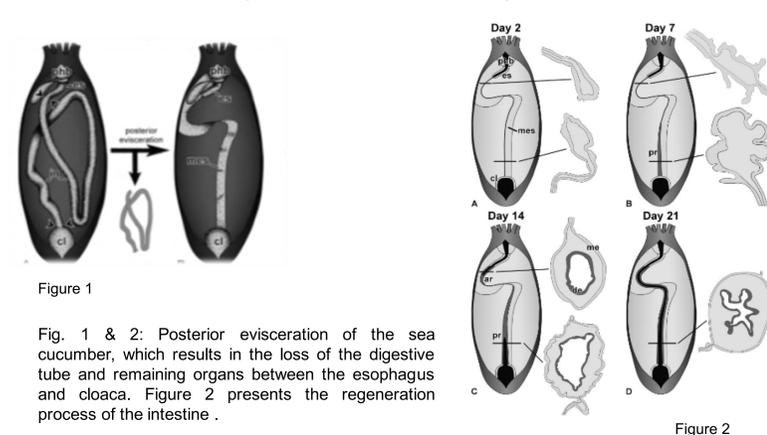


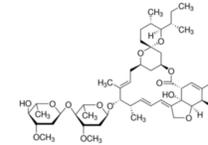
Figure 1

Fig. 1 & 2: Posterior evisceration of the sea cucumber, which results in the loss of the digestive tube and remaining organs between the esophagus and cloaca. Figure 2 presents the regeneration process of the intestine.

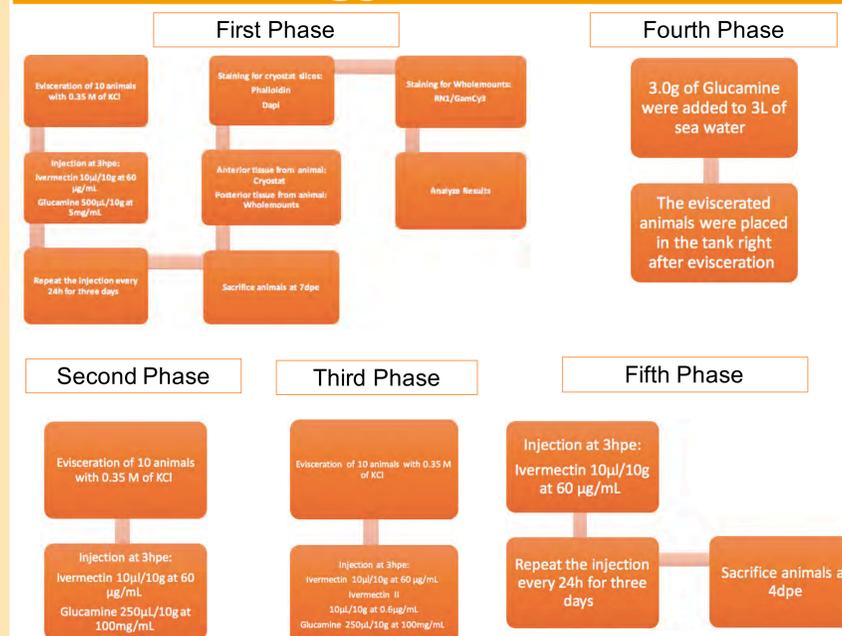
Figure 2

Purpose

- Determine if modulation of the GlyCl Channel has repercussions in the regenerative ability of the *Holothuria glaberrima's* intestine
- Via the administration of Ivermectin, the GlyCl Channel will be activated causing a hyperpolarization of the cell and ultimately inhibiting the action potential

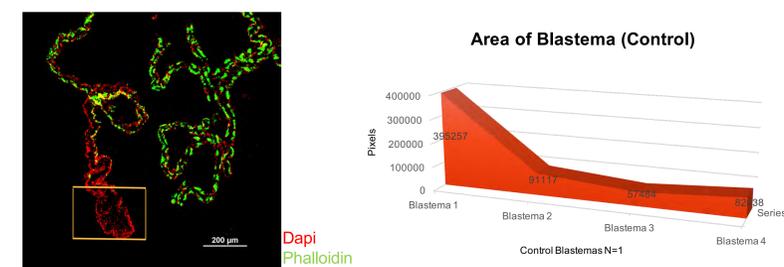


Methodology



Results

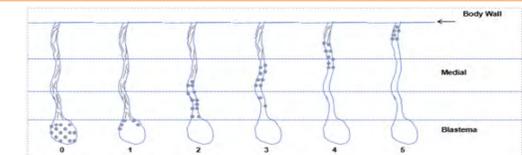
Area of the Blastema



The area of the *H. glaberrima's* blastema was measured as a way to identify if the hyperpolarization of the cells had any repercussions in the growth of the regenerating intestine

Results

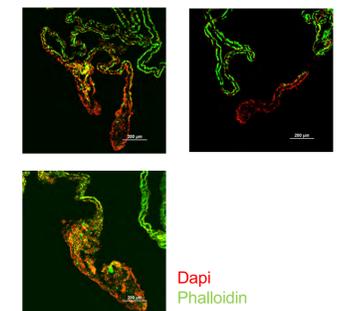
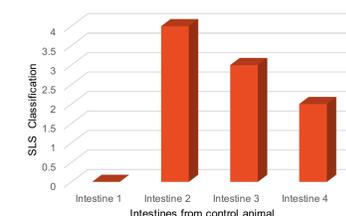
Spindle Like Structures (SLS) Classification System



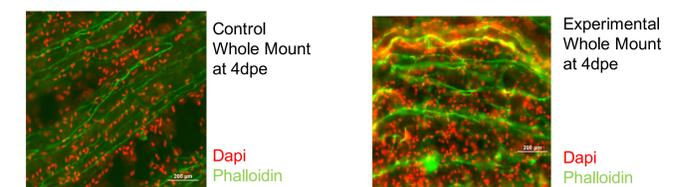
The classification of the SLS consists on identifying in which part of the regenerating intestine the Spindle Like Structures are present as well as numerically describe the progress of dedifferentiation. In this model we assign a numerical value to the position of the SLS in the regenerating intestine, the farther the SLS's are from the blastema, the more advanced is the dedifferentiation and the numerical value is higher.

Muscle Dedifferentiation by Localization of SLS

Muscle dedifferentiation by localization of SLS



Nervous Fiber Organization by Whole Mounts Technique



The Whole Mount technique will allow us to visualize the organization and morphology of the nervous fibers that are localized in the mesentery of the regenerating intestine.

Conclusions

- The N-Methyl-D-glucamine resulted to be fatal for the sea cucumber, other concentration or methods for the replacement of Na⁺ ions must be considered
- The animal model resisted the Ivermectin but couldn't last for up to 7dpe; other concentration must be explored to expand the survival time
- Preliminary results confirm that the histological and whole mount technique are working and ready for future experiments
- Preliminary results from whole mount indicate that the Ivermectin could possibly play a role in the organization of the nervous fibers