Downstream Targets of CART peptides in Mediating Regenerating Zebrafish Fin Folds

Regeneration is the ability to repair and replace damaged or lost tissue Some organisms like humans are unable to regenerate most damaged or lost body parts, whereas other organisms such as zebrafish and salamanders can. Regeneration allows organisms to repair sustained bodily damage that may otherwise be fatal if accumulated over time. Yet, the mechanism underlying this difference remains largely unknown. What is currently known about regeneration is that it involves several key factors: formation of an apical epithelial cap, recruitment of specialized cells to the wound site, and differentiation of proliferated stem cells (Kumar et al., 2007). In addition, classic studies have uncovered a key role of nerves in appendage regeneration. Specifically, for regeneration to occur intact nerves are required (Kumar et al., 2007) and that inadequate nerve supply in the site of the wound results in inhibited or no regeneration (Drachman and Singer, 1971). Unlike zebrafish and salamanders, the human capacity for regeneration is limited. This may be attributed to an evolutionary divergence that resulted in different regeneration pathways that may inhibit regeneration in humans (Hesse et al., 2015). However, an improved understanding of why and how some organisms can regenerate body parts, may facilitate development of improved medical treatments.

Zebrafish (*Danio rerio*) are an ideal model organism for studying regeneration. Zebrafish have short generation time, highly innervated tails, a fast rate of regeneration, and can be genetically modified to study functions of particular genes. In a pilot study, we investigated whether expression of Cocaine-and-amphetamine regulated transcript (CART) and Calcitonin gene-related peptide (CGRP) affected rates of regeneration. We found that increased expression of CGRP did not have a significant effect on regeneration. However, we found that increased expression of CART enhances the rate of regeneration of the larval zebrafish fin fold but the mechanism underlying this effect remains unknown.

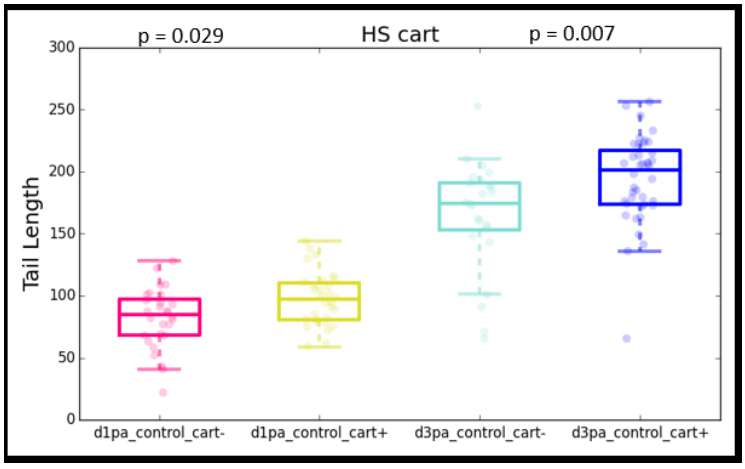

Figure 8. Quantification of Regeneration – HS CART Combined Data. There was a statistically significant difference in rate of regeneration in when comparing control fish to HS CART fish at both d1pa and d3pa. Statistical significance was determined using a Kruskal-Wallis Test.

To elucidate the connections between nerves and regeneration in zebrafish we are using pharmacological treatments that enhance or block nerve activity and how this may impact growth. We hypothesized that zebrafish subjected to pharmacological reagents that promote or

inhibit nervous system activity may differ in rate of regeneration. Previous experiments with pharmacological reagents known to decrease sensory neuron activity such as ethanol and tricaine methanesulfonate yielded results that suggested that a decrease in sensory neuron activity also decreased regeneration. In addition, pharmacological reagents that increased nervous system activity such as nicotine and caffeine were also tested but resulted in inconclusive data (Lantz-McPeak et al., 2015). We will investigate the role of these pharmacological reagents and how they may affect the rate of regeneration at various concentrations. We will also determine if these pharmacological reagents are affecting sensory neuron activity or function. Furthermore, we are investigating nerve associated genes known to be associated with regeneration including agr1, nrg1, ngn1, and duox. To date, we have successfully induced multiple mutations in each of these genes via the CRISPR-Cas9 system, and are actively working to cross these mutations to homozygosity. We are also investigating whether expression of these genes is regulated by CART, and whether the effects of increased CART persist when these genes are knocked out. In doing so we will be cloning and characterizing in larval amputated zebrafish known from other systems to be important in regeneration. I will present data about progress in generating CRISPR knockouts and examining expression of these genes in zebrafish with ubiquitous expression of CART.