Increased Neural Activity during Regeneration of Limbs in Newts

Linh Trang, Cyprian Weaver, BN Singh, Daniel J Garry

Lillehei Heart Institute
University of Minnesota

Abstract

Regeneration is a unique phenomenon that is robustly expressed in lower forms of life including the newt where it functions as an essential biological feature of life. As higher forms, we as mammals have developed a highly efficient immune system designed to repair rather than regenerate new tissues. This repair however is often connective tissue that no longer supports the parenchyma that it has replaced. Currently biomedical science is focusing considerable effort on the understanding and recovery of regenerative elements that could be applicable in a more effective mammalian response to injury. Some early research has shown by older methods that the brain may respond to regeneration and thus may have a role in regeneration. The present study was undertaken to assess changes in the brain that may in fact regulate by some unknown mechanism the dynamics underlying regeneration. We investigated protein expression in the brains of newts at various intervals of recovery following limb amputation. Subsequent to amputation and regeneration we found that the brain upregulates protein markers indicative of metabolic stress in specific nuclei of the newt brain not present in control animals. This analysis of the brain provides the basis of examining the expression of putative novel proteins specifically related to limb regeneration that may functionally link the central nervous system to regeneration which has not been shown to date.

Background

An early work has suggested increased protein synthesis in unidentified proteins of newt brains during limb regeneration (Tsonis 1992). However, these have not been identified or localized specifically in the brain. The aim of the present study was to further characterize the type of protein markers in response to limb amputation.

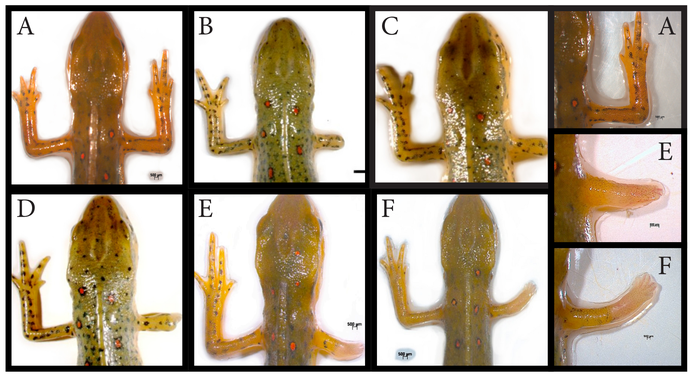

Figure 1: Newts undergoing limb regeneration.
(A) Uninjured control newt. (B) 1 week post-amputation. (C) 2 weeks post-amputation. (D) 3 weeks post-amputation. (E) 6 weeks post-amputation. Notice initiation of blood vessel formations for incoming paws. (F) 8 weeks post-amputation. Notice further development of paws.

Results

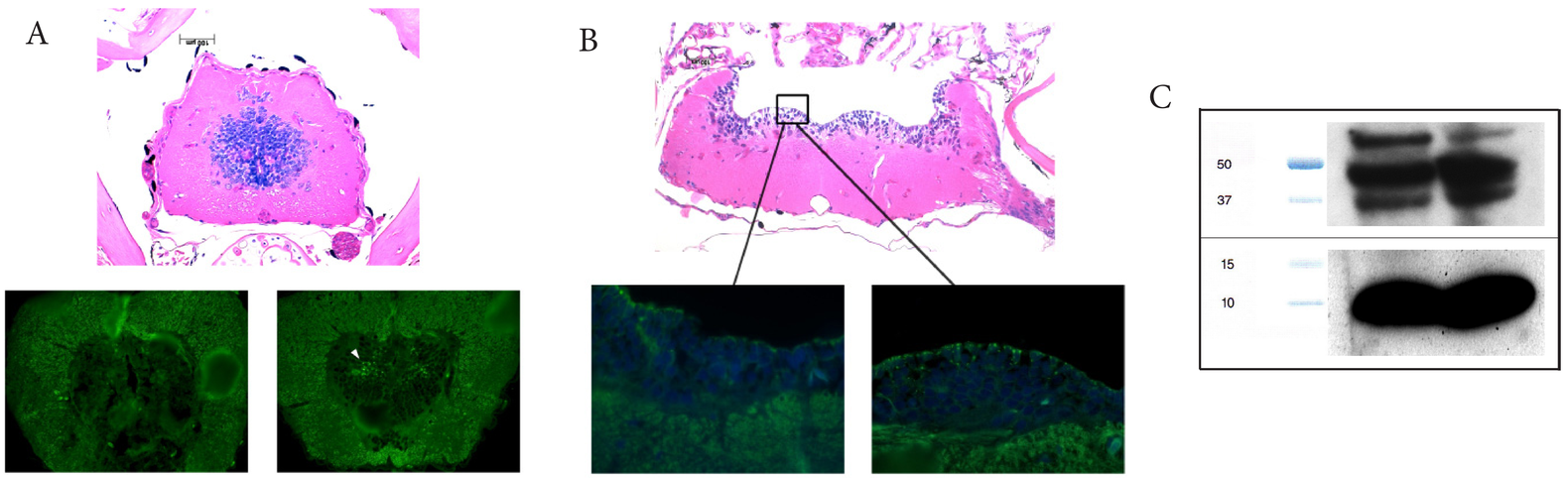

Figure 2.
(A) Newt brain sections taken at the spinal cord level with β-amyloid staining. Non-specific staining are artifactual in the control brain (left). Arrowhead indicates specific staining in the neurons of week 4 post-limb amputated newts (right).
(B) Newt brain sections taken at medullary level labeling neurons with β-amyloid in area acusticolateralis and underlying fibers of the tractus solitarius. The neuronal expression is strong in the two-week post amputated newt (right) in comparison to the intact newt (left).
(C)Western blot analysis with c-myc (top) and p38 (bottom) antibodies suggests a stronger protein expression as early as 2 weeks post-amputation (right well) than the intact control newt (left well).

Methods

- The forelimbs of 50 Eastern Red-Spotted Newts, Notophtalmus viridescens, were amputated proximal to the body following anesthesia in 0.1% MS-222 solution for 10 minutes.
- For histological analysis, control and limb amputated newt brains were isolated and prepared for cryosectioning. The tissues were then processed for immunohistochemistry using primary antibodies: c-myc, c-fos, β-amyloid, and Phos p-38. They were then incubated in respective fluorescence-labeled secondary antibodies and counterstained with DAPI to label nuclei.
- Control and week 2 post-amputated newt brains were sampled for protein detection, isolated, snap frozen in liquid nitrogen, and ground. The protein was processed and analyzed using western blot procedures to label protein according to molecular weight.

Heart Exploration

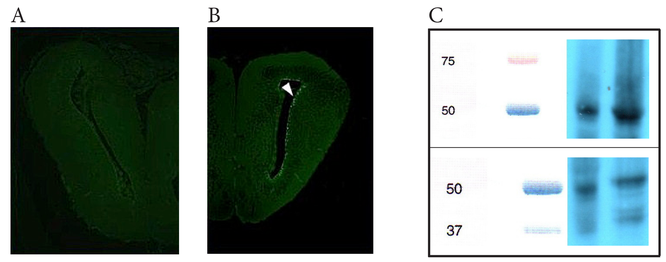

Figure 3.
(A) Control section of newt brain at the level of cerebral hemispheres using c-fos antibody.
(B) Experimental section of newt brain, using c-fos antibody, at the same level as control (left) from week 2 post-cardiac resection. Labeled cells are indicated by the arrowhead within the nucleus limitans.
(C) Western blot analysis with c-myc (top) and p38 (bottom) antibodies suggests a stronger protein expression as early as 2 weeks post-cardiac resection (right well) than the uninjured control newt (left well).

Discussion / Conclusion

Based on our results, we see a neural upregulation in the expression of our stress markers due to limb amputations in newts (Fig. 2). In addition, the preliminary data support protein expression as early as two weeks post-limb amputation (Fig. 2). Therefore, our findings indicate that the central nervous system does undergo some response and increase in activity as the limb regenerates. Furthermore, our histological comparison of brains of uninjured newts and newts regenerating from cardiac resection indicate a much stronger change in expression (Fig 3). The protein expression also seems to be intensified in cardiac resected newts (Fig. 3). This analysis of the brain provides the basis of examining the expression of putative novel proteins specifically related to limb regeneration that may functionally link the central nervous system to regeneration which has not been shown to date.