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Developmental and adult-specific processes contribute to *de novo* neuromuscular regeneration in the lizard tail

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ABSTRACT

Peripheral nerves exhibit robust regenerative capabilities in response to selective injury among amniotes, but the regeneration of entire muscle groups following volumetric muscle loss is limited in birds and mammals. In contrast, lizards possess the remarkable ability to regenerate extensive *de novo* muscle after tail loss. However, the mechanisms underlying reformation of the entire neuromuscular system in the regenerating lizard tail are not completely understood. We have tested whether the regeneration of the peripheral nerve and neuromuscular junctions (NMJs) recapitulate processes observed during normal neuromuscular development in the green anole, *Anolis carolinensis*. Our data confirm robust axonal outgrowth during early stages of tail regeneration and subsequent NMJ formation within weeks of autotomy. Interestingly, NMJs are overproduced as evidenced by a persistent increase in NMJ density 120 and 250 days post autotomy (DPA). Substantial Myelin Basic Protein (MBP) expression could also be detected along regenerating nerves indicating that the ability of Schwann cells to myelinate newly formed axons remained intact. Overall, our data suggest that the mechanism of *de novo* nerve and NMJ reformation parallel, in part, those observed during neuromuscular development. However, the prolonged increase in NMJ number and aberrant muscle differentiation hint at processes specific to the adult response. An examination of the coordinated exchange between peripheral nerves, Schwann cells, and newly synthesized muscle of the regenerating neuromuscular system may assist in the identification of candidate molecules that promote neuromuscular recovery in organisms incapable of a robust regenerative response.

1. Introduction

While salamanders, lizards, and teleost fish exhibit the capability to regrow entire appendages, the ability to regenerate complex, multi-tissue structures is limited in most mammals and birds (Bellairs and Bryant, 1985; Tsonis, 2000; Brockes and Kumar, 2005; Alibardi, 2014; Tanaka, 2016). Regeneration in urodele amphibians, such as the axolotl, has been the focus of research efforts, but studies in lizards could shed light on conserved genetic pathways as they are evolutionarily more closely related to mammals. Lizards such as the green anole, *Anolis carolinensis*, can readily self-amputate, or autotomize, their tails when threatened and regenerate a functional replacement (Cox, 1969; Gillis et al., 2009; Alibardi, 2014). Understanding the process of lizard tail regeneration may assist in discovering relevant cellular and molecular mediators of appendage regeneration in other amniotes such as humans.

The regeneration of a functional appendage requires dynamic and tightly regulated interactions between signaling centers, stem cell progenitors, and differentiated cell types (Sharma and Belmonte, 2001; Murphy et al., 2011; Reddien and Tanaka, 2016). An important goal has been to define the specific cellular subtypes and molecular signals that direct appendage regrowth (Tornini and Poss, 2014; Reddien and Tanaka, 2016). The regeneration of the neuromuscular system in the lizard tail is crucial for restoring appendage functionality and has been recognized for some time (Hughes and New, 1959; Bellairs and Bryant, 1985; Alibardi, 2014). The peripheral nerves comprised of axons and Schwann cells readily regrow following injury in both mammals and lizards and provide critical molecular cues that promote regeneration of other cell types (Hughes and New, 1959; Singer, 1961; Bosse et al., 2006; Pirotte et al., 2016). Birth dating experiments suggest that regenerating axons in the lizard tail are not derived from newly born neurons

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(Duffy et al., 1992; Alibardi et al., 1992). Instead, retrograde labeling experiments have shown that most of the newly formed axons in the tail are derived from sprouting of axotomized dorsal root ganglia (DRG) and spinal motor neurons rostral to the breakpoint (Hughes and New, 1959; Duffy et al., 1992; Cristino et al., 2000a, 2000b, 2000c). The expansion in the peripheral innervation