UNIVERSITY OF CALIFORNIA, SAN DIEGO

Investigating Neuro-Muscular Junction Formation During Naturally Occurring Muscle Loss in the Neonatal Jerboa Foot

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

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The Thesis of Michelle Dasiree Flores is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chapter 1 is Co-authored by Michelle Flores, Hannah Grunwald, Dr. Rio Tsutsumi, Mai Tran and Joel Erberich. Mai and I jointly performed the RNA *in situ* hybridizations for this project. Mai and I also worked on the initial qualitative observations of the timing of muscle loss through immunofluorescent detection of myosin heavy chain. As I moved forward with other aspects of my thesis project Mai and Joel continued to quantify the rate of muscle loss by counting individual myofibers in cross sections of jerboa tissue.

ABSTRACT OF THE THESIS

Investigating Neuro-Muscular Junction Formation During Naturally Occurring Muscle Loss in the Neonatal Jerboa Foot

by

Michelle Dasiree Flores

Master of Science in Biology

University of California, San Diego, 2017

Professor Kimberly Cooper, Chair

Professor James Posakony, Co-Chair

The limb is one of the most evolutionarily diverse structures in vertebrate morphology. Our lab focuses on the lesser Egyptian jerboa, *Jaculus jaculus,* as a opportunity to identify the mechanisms that reshape the limb as a consequence of natural selection. The jerboa is a bipedal rodent with elongated hindlimbs and three toes on feet that lack intrinsic foot muscles. During my thesis work, I sought to understand the progression and mechanism of muscle loss in the context of its relationship with the motor nerve. We initially tested two alternate hypotheses: either jerboas never develop foot muscles or muscle fibers form and are lost over time. RNA *in situ* hybridization to *MyoD* and immunofluorescence detection of myosin heavy chain determined that jerboas are born with muscles in their feet, which are virtually nonexistent by post-natal day 7. I examined the structure of the nerve and its associated neuromuscular junctions to evaluate if proper innervation occurs at early critical stages and if that innervation is maintained during the earliest stages of myofiber degeneration. I utilized immunohistochemistry to examine the integrity of the nerve structure and to identify structural synapses. I also injected neonatal jerboas with CTB-555 labeling dye to retrograde trace motor neurons of the foot back to their origin in the spinal cord. I discovered that the muscles in the feet of the jerboa receive proper innervation and that this innervation is maintained for several days after birth, even after the myofibers begin to disorganize and degenerate. These results indicate it is unlikely a developmental innervation failure is responsible for muscle loss in the jerboa but rather itself degenerates concurrent with loss of its innervation target.

INTRODUCTION

The mechanisms of musculoskeletal development that are shared across various species are well understood. However, we are only just beginning to understand how diversity has arisen between species. The lesser Egyptian jerboa, *Jaculus jaculus,* provides an opportunity to gain insight into the cellular and molecular mechanisms driving the evolution of limb development. The jerboa is an obligate bipedal rodent with a distinct morphology from its closest quadrupedal relative, the birch mouse (Moore, 2015). While the forelimb is "mouse-like", the jerboa hind limb is greatly elongated with fused central metatarsals and three toes (Cooper, 2011). Jerboas have lost the ability to grasp and climb vegetation and have also lost the muscles in their feet that spread the toes for climbing in quadrupedal relatives (Figure 1).

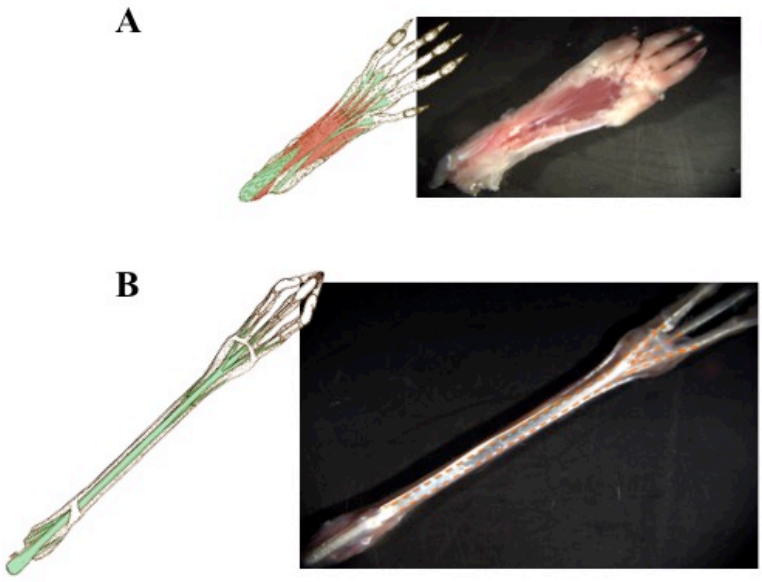

Figure 1: schematic and gross anatomy of the mouse foot (a) and jerboa foot (b). Image provided by Howell, AB. The Williams and Wilkins Company, 1926.

The lesser Egyptian jerboa, *Jaculus jaculus,* is only 50 million years divergent from *Mus musculus* and is easily reared in the laboratory, making them an exceptional model for comparative developmental and molecular analyses (Cooper, 2011).

The jerboa foot functions as a simple lever mechanism comprised of bone, skin, and connective tissue. The jerboa bounds across the deserts of its native Mongolia, China, and Egypt evading predatory animals with its unique gait. Saltatorial organisms only require the extrinsic muscles necessary for extension and flexion of the feet, as their range of movement is limited to one plane. Jerboas are among saltatorial organisms that have lost most of the adductive and abductive muscles that are intrinsic to the feet and are required for rotational movement. (Howell, 1932; Berman,1985). We are interested in understanding the molecular and cellular mechanisms that contribute to the striking phenotype of muscle loss in the jerboa foot in an evolutionary context. A better understanding of the fundamental mechanisms of muscle maturation and maintenance may eventually lead to the generation of therapies to mitigate undesired muscle loss through manipulation of these mechanisms.

We reasoned that the jerboa intrinsic foot muscles may be evolutionarily lost in the adult through one of two developmental mechanisms: either muscle progenitor cells are never present in the developing embryonic foot because of early defects in specification or migration, or muscle initially forms normally and is subsequently lost.

Skeletal muscle cells produced by the somites originate from the dermomyotome, of the dorsal somite, an epithelial structure positioned between the neural tube and somatopleural mesoderm (Christ, 1972; Schmidt, 1998). During embryogenesis, myogenic precursor cells invade the limb mesenchyme and subsequently begin to

aggregate and differentiate into dorsal and ventral premuscular masses (Christ et al, 1977). As the limb bud grows, migrating pools of muscle progenitors become subdivided into dorsal and ventral muscle masses.

In time, each muscle mass separates to form individual, anatomically distinct muscles associated with the upper arm/leg (stylopod), lower arm/leg (zeugopod), and hand/foot (autopod). The timing of development and differentiation progresses from proximal to distal such that the muscles of the hands and feet are the last to form (Christ, 2002). The premuscle masses in the limbs are comprised of two parts: a superficial layer of *Pax3* and *Myf5* expressing proliferating muscle precursor cells and a deep layer in which differentiating myoblasts express MyoD and muscle proteins (Christ, 2002). The activation of muscle determination factors MyoD, Myogenin and MRF-4 in response to local signals initiates myogenesis in the limb bud (Figure 2); it is only once cells migrating from the somite reach the limb that they begin to express these muscle specification genes (Tajbakhsh & Buckingham, 1994). While mice mutant for either MyoD (Rudnicki et al, 1992) or *Myf5* (Braun et al., 1992) display no clear defects in muscle differentiation, double mutants lack all skeletal muscle (Rudnicki et al, 1993). Without these factors, cells that would normally become myoblasts migrate aberrantly and adopt non-muscle cell fates (Tajbakhsh et al., 1996).

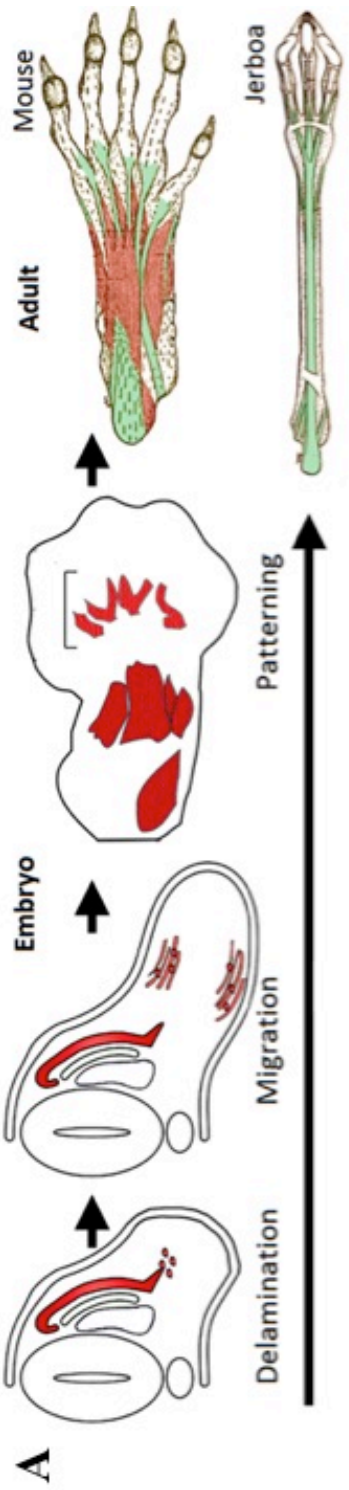

Figure 2: Schematic depicting myogenesis. Modified from Howell AB 1926.

Sonic Hedgehog is a secreted molecule expressed in the zone of polarizing activity (ZPA) that influences patterning and development of multiple limb tissues, including skeletal muscles (Hu et. al, 2012). In the absence of Shh activity there is a loss of limb musculature, with the exception of a small portion of the dorsal muscle adjacent to the humerus (Kruger et al. 2001). Explant cultures indicate that this loss could be due to a requirement for Shh to maintain the expression of *Myf5* and MyoD (Kruger et al. 2001).

In order to distinguish the direct and indirect effects of Shh on limb muscle development, Hu and colleagues evaluated the cell-autonomous and non-cell-autonomous functions of Shh signaling during limb muscle development using a muscle-specific *Smoothened (Smo)* knockout (Hu et al. 2012). They found that Shh acts non-autonomously in lateral plate mesoderm-derived tissues to pattern limb musculature. They also found that Hh signaling is required cell-autonomously to maintain cell survival in the dermomyotome, to initiate *Myf5* and MyoD expression in the ventral limb muscle mass, and to promote progenitor migration into the distal limb. Muscle-specific *Smoothened* knockout mice lack autopod muscles (Hu et al. 2012). This phenotype inspired us to determine whether evolutionary loss of muscle in the jerboa foot might be due to a similar early defect in specification or migration of muscle progenitor cells, or if muscle initially forms normally and is subsequently loss through muscle atrophy.

Atrophy is defined as a decrease in the size of a tissue or organ due to cellular shrinkage caused by the loss of organelles, cytoplasm, and proteins (Bonaldo and Sandri, 2013). Muscle atrophy occurs when protein degradation rates exceed protein

synthesis (Sandri, 2013). Intrinsic factors, such as muscle degradation pathways and extrinsic factors, such as muscle injury and denervation, can result in atrophy of skeletal muscle.

Two major protein degradation pathways, the ubiquitin-proteasome and the autophagy-lysosome systems, are known to become activated during muscle atrophy and contribute to the loss of muscle mass (Sandri, 2013). In muscle, the ubiquitin-proteasome system is required to remove sarcomeric proteins upon decrease in muscle activity (Bonaldo and Sandri, 2013). A loss in muscle mass is associated with increased conjugation of ubiquitin to muscle proteins, increased proteasomal ATP-dependent activity, increased protein breakdown, and upregulation of transcripts encoding ubiquitin-proteasome pathway components (Bonaldo and Sandri, 2013). The autophagy-lyososome system in skeletal muscle is crucial because alterations to this system contribute to the pathogenesis of several genetic muscle diseases (Bonaldo and Sandri, 2013). Evidence shows that lysosomal degradation contributes to protein breakdown in denervated muscle (Furuno et al., 1990), and ablation of Atg7, an enzyme of autophagic machinery, causes disorganized sarcomeres and activation of the unfolded protein response, which triggers myofiber degeneration (Masiero et al., 2009). These two pathways play an integral role in regulating overall muscle homeostasis.

Extrinsic factors that lead to muscle atrophy include denervation, fasting, and mechanical stress. Denervation-induced atrophy leads to a final state in which the functioning muscle tissue is largely replaced by fibrous connective tissue and fat, as well as scattered attenuated muscle fibers (Carlson, 2014). As a denervated muscle

proceeds towards terminal atrophy, it passes through three identifiable stages (Carlson, 2014). During the first stage, there is an immediate loss of function and muscle fiber atrophy brought on by section of the nerve. The second stage is characterized by severe muscle atrophy, including the disorganization of sarcomeric proteins (Carlson, 2014). During the long third phase, interstitial fibrosis and the appearance of adipocytes dominate the tissue structure (Carlson, 2014). Overall, the number of remaining muscle fibers is greatly diminished and what is left bears little resemblance to normal fibers.

The first goal of the project detailed here in my Master's thesis was to identify the point in development at which muscle development fails in the jerboa foot. Once we determined myofibers are lost after their initial differentiation, my goal was to investigate whether innervation failure might contribute.

CHAPTER 1

We first tested the hypothesis that muscle progenitor cells fail to migrate into the distal limb bud, which would prevent the earliest stages of autopod myogenesis. If muscle loss in the jerboa foot were due to an early migration or patterning defect, we would expect to see an absence of early muscle progenitor cells in the embryonic foot or a failure of these cells to correctly organize into muscle bundles. Alternatively, if muscle progenitor cells migrate into the distal limb and successfully initiate myogenesis, this would suggest a later failure of muscle differentiation or maintenance.

We performed Whole-mount RNA *in-situ h*ybridization to identify migrating foot muscle progenitor cells by their expression of MyoD mRNA, a transcription factor that is critical for early stages of muscle differentiation. MyoD mRNA expression was identified in the distal hind limb bud of the embryonic jerboa in close association with the developing digits (Figure 3). This expression pattern is consistent with the mouse fore- and hind limb and the jerboa forelimb, where muscle persists.

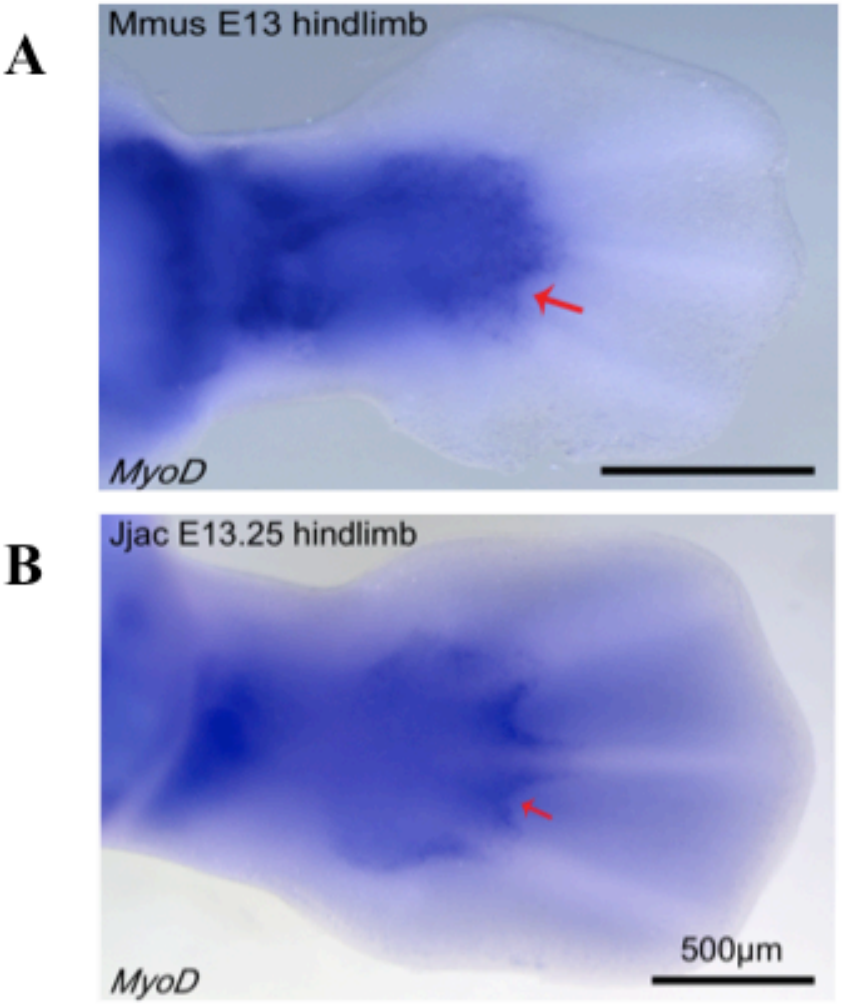

Figure 3: MyoD mRNA expression in the embryonic limb bud of mouse (a) and jerboa (b). Arrows indicate MyoD expression in the autopod.

These results demonstrate that muscle progenitor cells successfully migrate from the limb-level somite into the distal limb bud to occupy the appropriate locations to form the intrinsic foot muscles, This result suggests that muscle loss is not due to a failure of early migration or pattern. We next sought to determine whether the muscle progenitor cells initiate primary myogenesis to form multinucleated myofibers and, if so, at what stage of development these myofibers are lost.

To determine the timing of muscle loss, we examined the expression of myosin heavy chain, a contractile protein expressed in differentiated muscle, at late fetal and early postnatal stages of jerboa development by immunofluorescence (Figure 4). We discovered that neonatal jerboas have well-organized myofibers and that myofiber loss is initiated post-natally. We were able to identify two main sets of muscle groups, the superficial digital flexors that appeared more ventrally and the deeper interosseous muscles that lie along the plantar surface of the metatarsals. The deep digital flexor muscles never form in the jerboa foot, and robust tendon in their place partitions the remaining muscle groups.

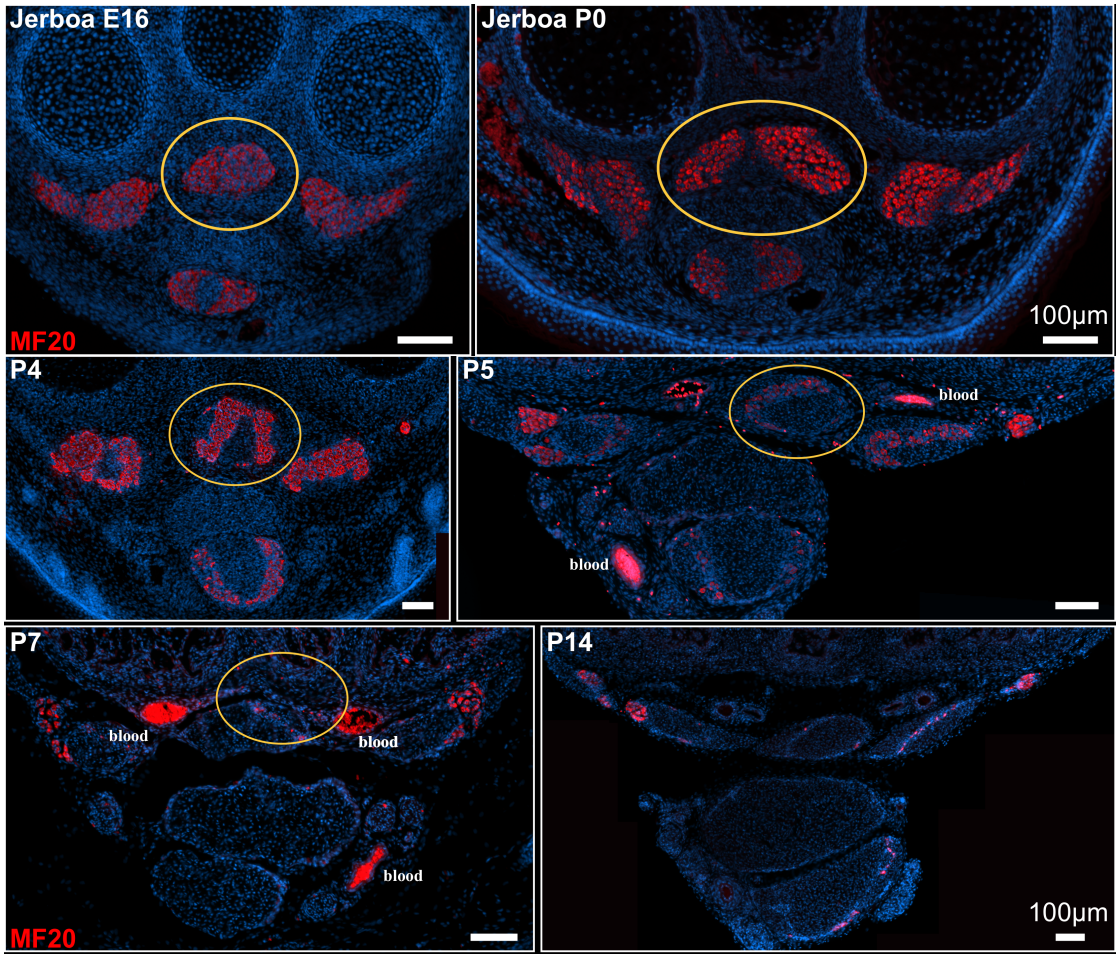

Figure 4: Immunohistochemistry of third interosseous muscle in jerboa. Myofibers (myosin) shown in red, DAPI shown in blue.

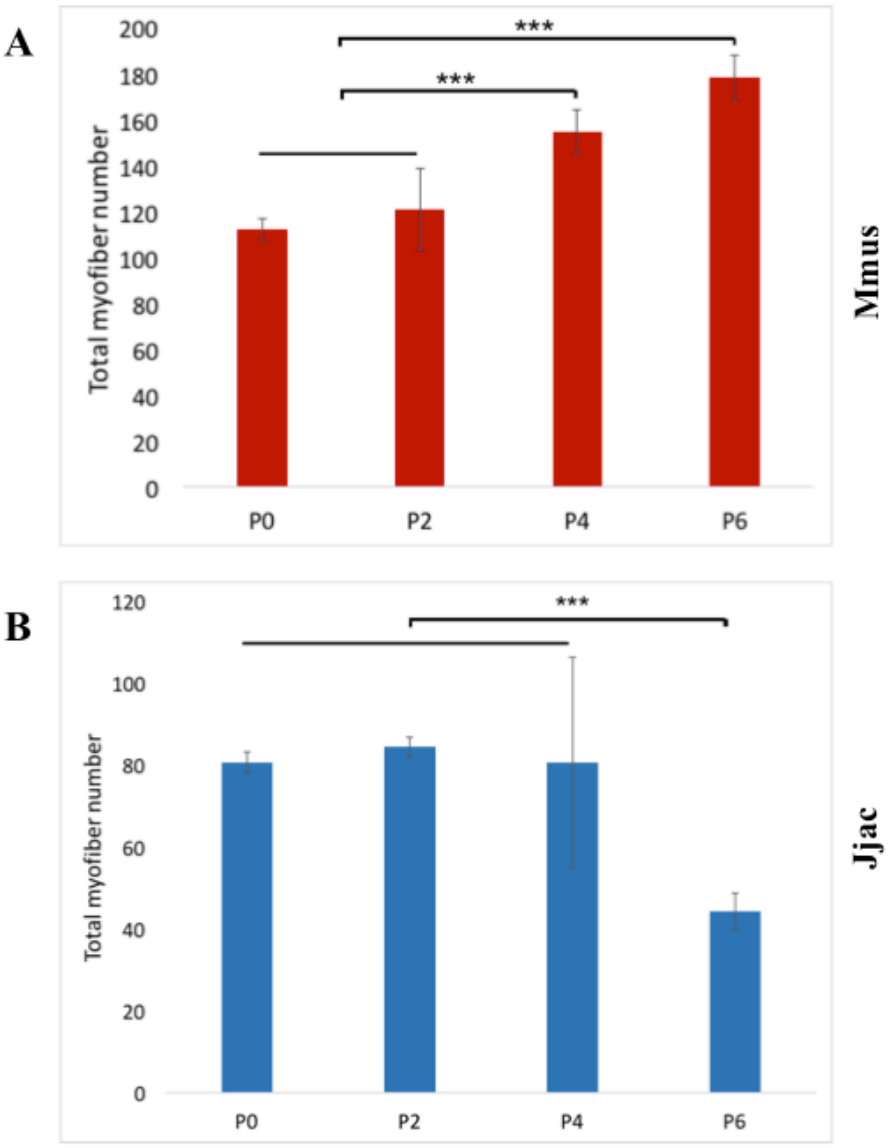

Figure 5: From P0 to P6 there is a steady increase in the number of myofibers seen in the third interosseous muscle of the mouse (A), whereas in the jerboa the number of myofibers remains relatively stable until they begin to decrease between P4 and P6 (B).

Almost all intrinsic foot myofibers are lost by post-natal day seven (P7) (figure 4). An undergraduate student in the lab, Joel Erberich, later completed a quantitative assessment of muscle development in mouse and jerboa. This analysis revealed that in neonatal mice, myofiber count increases from birth to P6 (Fig5a), whereas in jerboas there is a rapid decrease in myofiber count that initiates between P4 and P6 (Fig5b). Together, these observations allowed us to pinpoint meaningful stages of development to test hypotheses with respect to the mechanism of muscle loss.

Chapter 1 is Co-authored by Michelle Flores, Hannah Grunwald, Dr. Rio Tsutsumi, sMai Tran and Joel Erberich. Mai and I jointly performed the RNA *in situ* hybridizations for this project. Mai and I also worked on the initial qualitative observations of the timing of muscle loss through immunofluorescent detection of myosin heavy chain. As I moved forward with other aspects of my thesis project Mai and Joel continued to quantify the rate of muscle loss by counting individual myofibers in cross sections of jerboa tissue.

CHAPTER 2

Muscle atrophy occurs due to lack of physical activity, disease, injury, or temporary disability. Muscle wasting is also characteristic of muscular dystrophies, a class of genetic myopathies characterized by varying degrees of myofiber necrosis, accumulation of connective tissue, and infiltration of the muscles by macrophages (Engel, 1986). In these disorders, the ultimate fate of the skeletal muscle is to undergo degeneration (Ontell, 1986). While muscle structural protein mutations are the most common cause of muscular dystrophies, such as dystrophin mutations that cause Duchenne muscular dystrophy, other disorders such as Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy are caused by the progressive degeneration of motor neurons.

The survival of skeletal muscle is, in part, dependent on signals provided by motor neurons (Carlson, 2014). The neuromuscular junction is a chemical synapse formed by the contact between a motor neuron and a muscle fiber. The nerve transmits a signal to the muscle fiber at the neuromuscular junction, causing muscle contraction (Pun, 2002). In the absence of innervation and activity, skeletal muscle undergoes an irrevocable development of atrophy, resulting in functional muscle tissue being replaced by fibrous connective tissue and fat (Carlson, 2014). As a denervated muscle progresses towards terminal atrophy, it passes through several characteristic stages; in rats, the stages are measured in terms of months, whereas human muscle requires years to fully atrophy (Carlson, 2014).

While loss of muscle mass after denervation in the adult occurs over a much longer time period than in the neonatal jerboa, we hypothesized that an initial failure or severely delayed innervation of the jerboa foot could contribute to muscle loss. I performed immunofluorescence stains at multiple stages of neonatal jerboa development to assess the integrity of the nerve and to verify if the structural synapse is intact over time. For these experiments, I focused in on the third interosseus muscle, because it demonstrates the least amount of variation between and within samples. I examined the expression of pre- and post-synaptic proteins, as well as Neurofilament protein to mark the axons. Slides were additionally co-stained with Titin, a sarcomeric protein that allowed for the visualization of the myofiber.

We identified a slight heterochrony in the innervation of mouse hind limb compared to the forelimb (Figure 6). This developmental delay was also observed in jerboa samples (Figure 7 and Figure 8). Therefore the intrinsic hind foot muscle morphology of the jerboa more closely resembles the morphology of the mouse intrinsic hind foot rather than that of the jerboa hand.

P0 Mouse Hand

P0 Mouse Foot

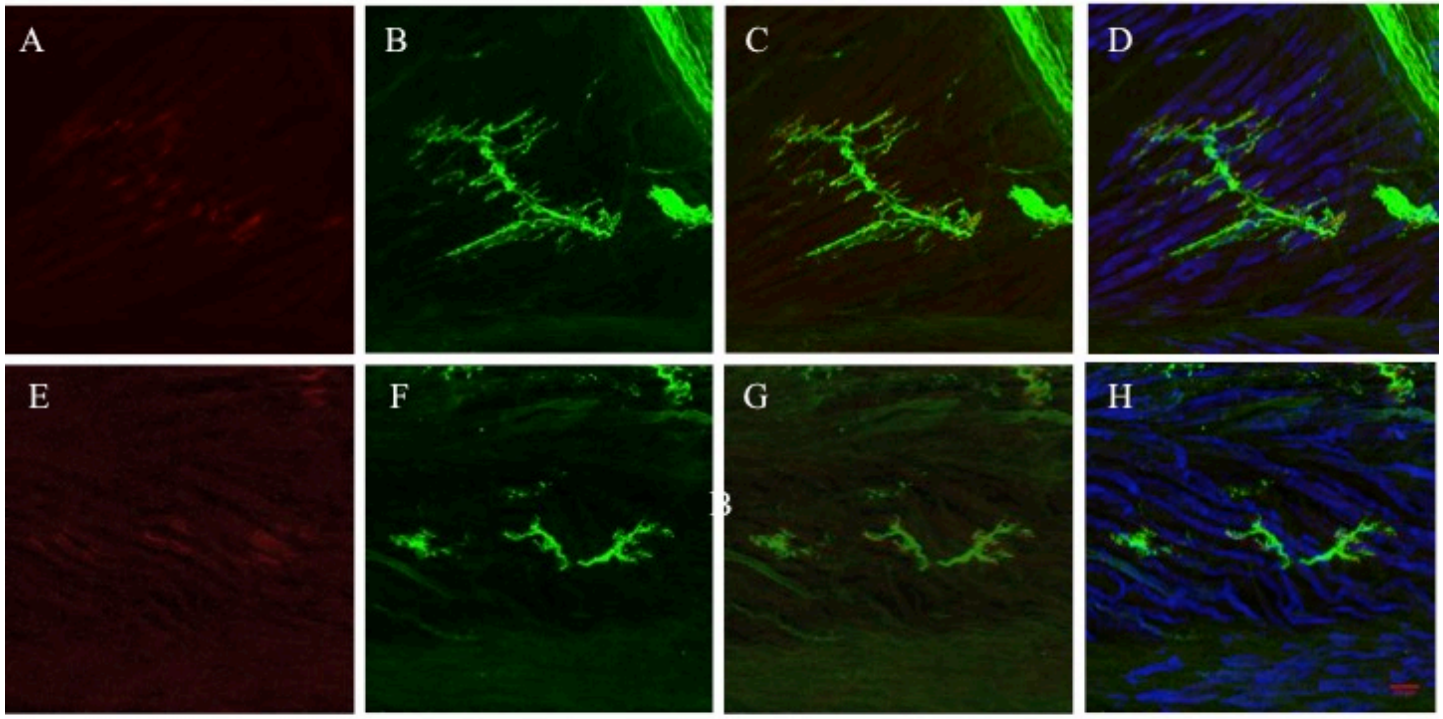

Figure 6: Innervation of the hand (A-D) and foot (E-H) muscle of a P0 mouse at the NMJ. AChR clusters labeled with α-Bungarotoxin (red) (a and e). Pre-synaptic markers are labeled with Synaptophysin (green), a pre-synaptic vesicle protein and axon filament is labeled with Neurofilment (green) (b and f). Pre-synaptic markers are juxtaposed and co-localized (yellow) with post-synaptic markers (c and g). Merge of previous panels with the addition of Titin (blue), a sarcomeric protein (d and h).
There is a slight heterochrony between the hand and foot.

Jjac P0 Hand

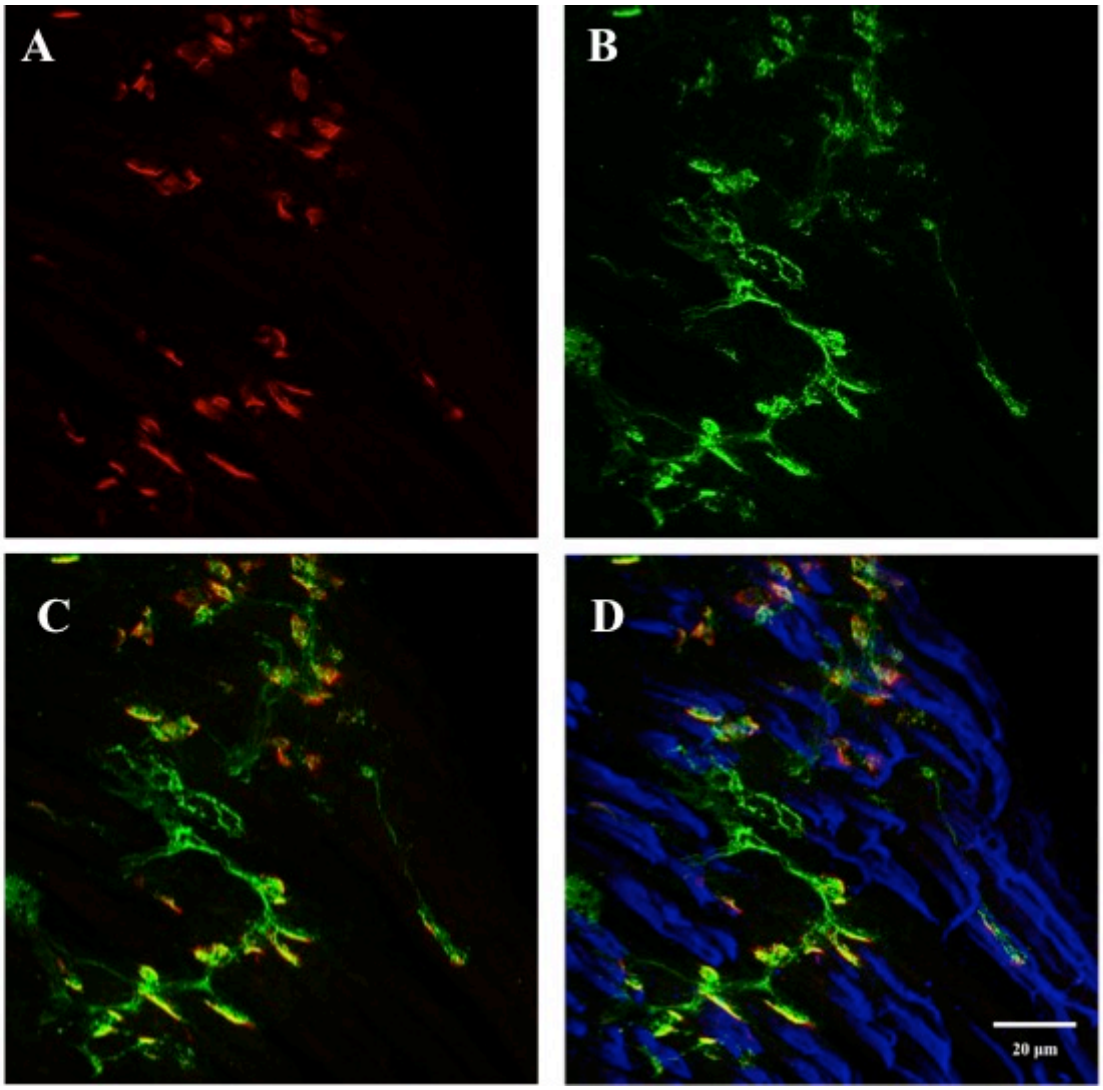

Figure 7: Innervation of the hand muscle of a P0 jerboa at the NMJ. AChR clusters labeled with α-Bungarotoxin (red) appear robust (a). Pre-synaptic markers are labeled with Synaptophysin (green), a pre-synaptic vesicle protein and axon filament is labeled with Neurofilment (green) (b). Pre-synaptic markers are juxtaposed and co-localized (yellow) with post-synaptic markers (c). Merge of previous panels with the addition of Titin (blue), a sarcomeric protein (d).

Jjac P0 Foot

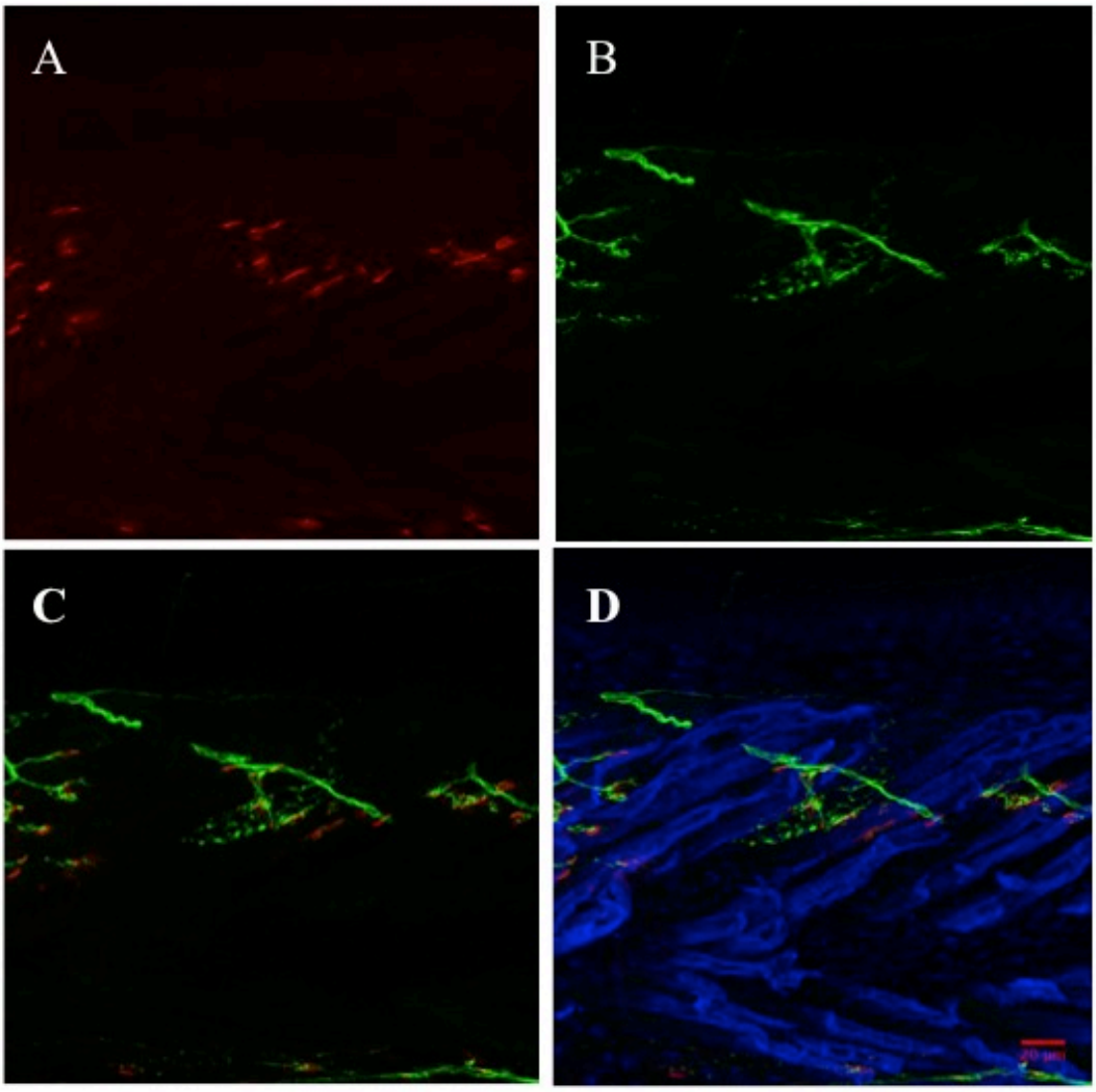

Figure 8: Innervation of third interosseous muscle at the NMJ of P0 jerboa. AChR clusters shown in red (a). Neurofilament and Synaptophysin shown in green (b). Merge of (a) and (b) in (c). Juxtaposition appears yellow. Panel (c) shown with Titin (blue) (d).

In the P0 jerboa foot muscle pre-synaptic and post-synaptic markers appear juxtaposed and are clearly associated with a myofiber, indicating proper formation of the structural synapse (Figure 8). We were unable to perform electrophysiological experiments within the scope of this project to determine if the nerve is physiologically active. In mice, acetylcholine receptors cluster in the absence of neural innervation, however this clustering is refined once the nerve makes contact with the muscle to align the AChR clusters with the path of the nerve (Arber et al., 2001). Furthermore, AChR clusters become more refined in response to chemical synapse activity as nonspecific clusters are eliminated over the course of several days during embryonic development. The refinement of the AChR clusters serves as a proxy for proper function, because this refinement will only occur in response to focal signals provided by motor axon terminals. In the jerboa intrinsic foot muscle at birth, synapses are intact with virtually no vacant Acetylcholine clusters (Figure 8).

As indicated in the previous chapter, myofibers start to become disorganized at postnatal day 4 and are almost entirely lost by postnatal day 7. At post-natal day 4, synapses in the jerboa foot appear thinner and more sparse compared to P0 synapses. There are also more vacant AChR clusters compared to P0 jerboa (Figure 9). This phenotype coincides with the disorganization of myofibers at the same developmental stage, suggesting that nerve and muscle degeneration are occurring concurrently.

Figure 9: Innervation of the hand (A-D) and foot (E-H) muscle of a P4 jerboa at the NMJ. AChR clusters labeled with α-Bungarotoxin (red) (a and e). Pre-synaptic markers are labeled with Synaptophysin (green), a pre-synaptic vesicle protein and axon filament is labeled with Neurofilment (green) (b and f). Pre-synaptic markers are juxtaposed and co-localized (yellow) with post-synaptic markers (c and g). Merge of previous panels with the addition of Titin (blue), a sarcomeric protein (d and h).

Specific subsets of motor nuclei in the spinal cord at the level of the fifth lumbar vertebra are associated with groups of muscles within the limb (Mendelsohn, 2017). We hypothesized that in the jerboa, if the integrity of the nerve innervating the foot muscles was not maintained, we would be unsuccessful in attempting to backfill neurons in the foot to their origin in the spinal cord. Utilizing a retrograde labeling dye, CTB-555, we were able to successfully backfill motor neurons in the foot to their origin in the spinal cord in neonatal jerboas (Figure 10). Jerboas injected at P0 and collected at P2 showed labeling of motor neurons in the spinal cord at lumbar position 5. Without intact axons, we would not expect to see cell bodies labeled in the spinal cord. These results indicate that the axonal projections were sufficiently intact at the time of labeling to be able to successfully backfill to the spinal cord, further corroborating the presence of intact neural projections in early neonatal jerboas.

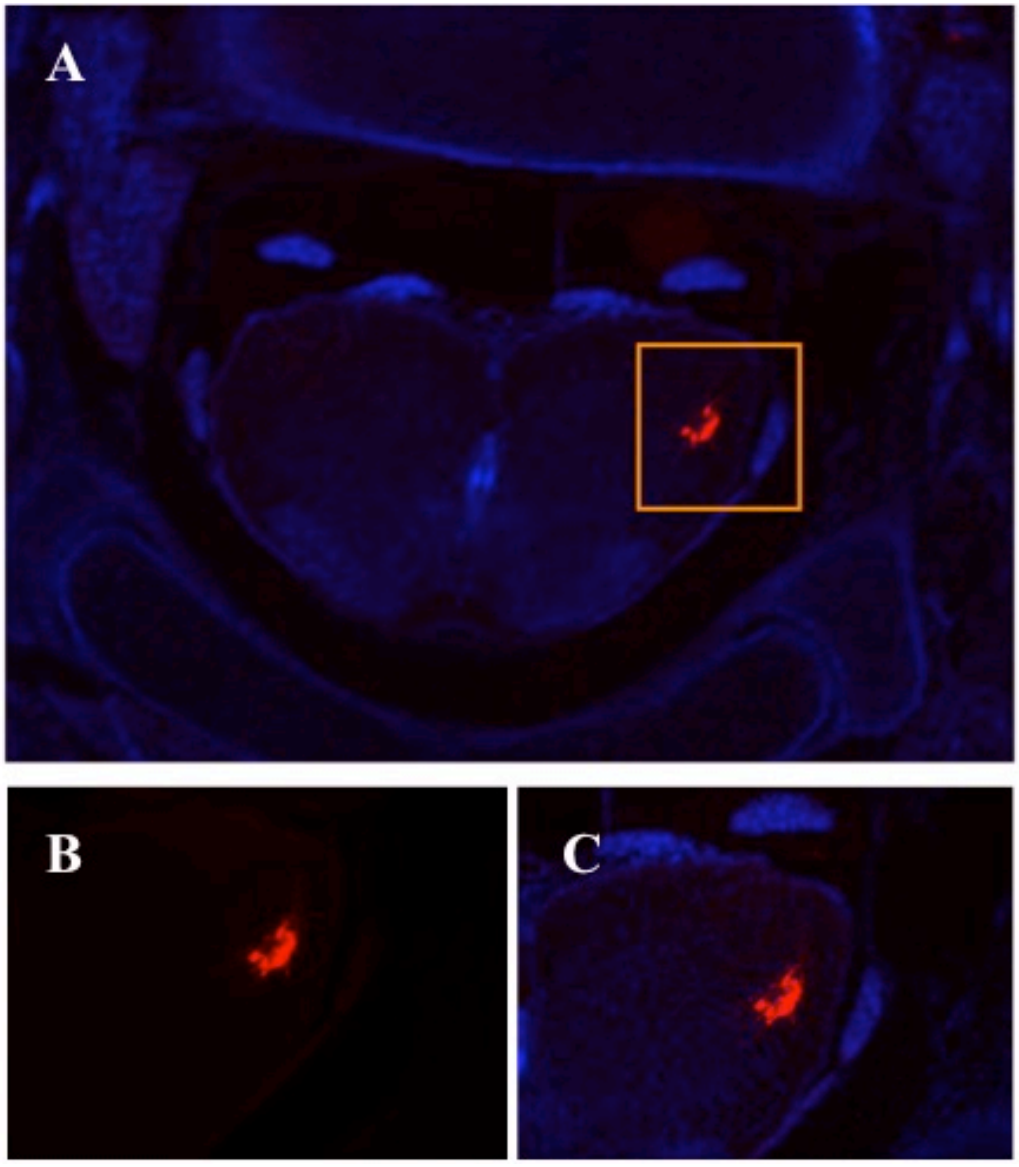

Figure 10: At post-natal day 2, motor neurons are successfully labeled at lumbar vertebrae 5 at 4x (a). Insert of panel A at 10x shown without DAPI (b) and with DAPI (c).

While there is ample evidence from the literature for the timing and progression of muscle degeneration after denervation in adults, there is less support for the effects of denervation in neonates. In severe SMA mice, one of the earliest detectable morphological defects is the loss of synapse occupation by motor axons (McGovern et al, 2008). As in the jerboa, motor axon formation in the severe SMA mouse model is initially normal with motor axons developing and extending to contact the appropriate muscles by E12.5 Denervation at the neuromuscular junction evidenced by unoccupied AChR clusters are detectable at embryonic day 18.5. (McGovern et al, 2008). According to images presented but not discussed in the literature, muscle persists until at least post-natal day 3 in these severe SMA mice (McGovern et al., 2008).

The homeobox gene HB9 (Harrison et al., 1994; Ross et al., 1998) is a selective marker of motor neurons in the developing spinal cord (Tanabe et al., 1998). In HB9 mutant mice, motor neurons lose differentiated properties (Arber et al, 1999; Thaler et al., 1999), and the phrenic nerve, which innervates the diaphragm muscle, fails to form during fetal development (Thaler et al., 1999). However, while HB9 mutant mice die shortly after birth with uninflated lungs, due to lack of innervation of the diaphragm muscle, the muscles themselves appear to form normally (Arber et al., 1999). Together, our observations of intact structural synapses and contiguous axons from the cell bodies to the muscle indicate the nerve is intact during early neonatal development. While it is unclear from the literature how long an early developing muscle persists after denervation, it seems likely the concurrent denervation during jerboa foot muscle degeneration is causative of myofiber loss.

CHAPTER 3: Summary and Future Directions

When muscle loss occurs due to injury or disease, it is expected that the motor axons will retract and the cell bodies in the spinal cord will die (Tovar-y-Romo et al, 2014). However, it is unclear if neurodegeneration also occurs in the context of evolutionary re-patterning or if nerve tracts are co-opted to innervate other muscles.

The natural and early loss of muscle in the jerboa foot offers a unique opportunity to test whether evolutionary and developmental neuroplasticity allows the nervous system to "re-purpose" cells by rerouting motor axons following the loss of skeletal muscle. We decided to fill intrinsic foot motor neurons of the foot to their origin in the spinal cord by intramuscular injection of fluorescently conjugated cholera toxin subunit b (CTB-Alexa555) and then allowed these animals to continue to develop to an age after all intrinsic foot muscle is lost. This experiment allowed us to test two alternative hypotheses: in the absence of foot muscles, the motor axons reroute to innervate more proximal muscles, or the entire foot motor pool in the spinal cord dies and is absent from adults.

As a control for our ability to label neurons, we have successfully filled mouse foot neuronal cell bodies by injecting CTB-555 into the foot muscle of neonatal mice. Since previous literature does not indicate how long the fluorescent label persists, I injected mice at postnatal day one and collected animals at a series of time points. From a series of collections at 3-4 day intervals, I was able to determine that dye fluorescence persists for 10 days after injection (Figure 11).

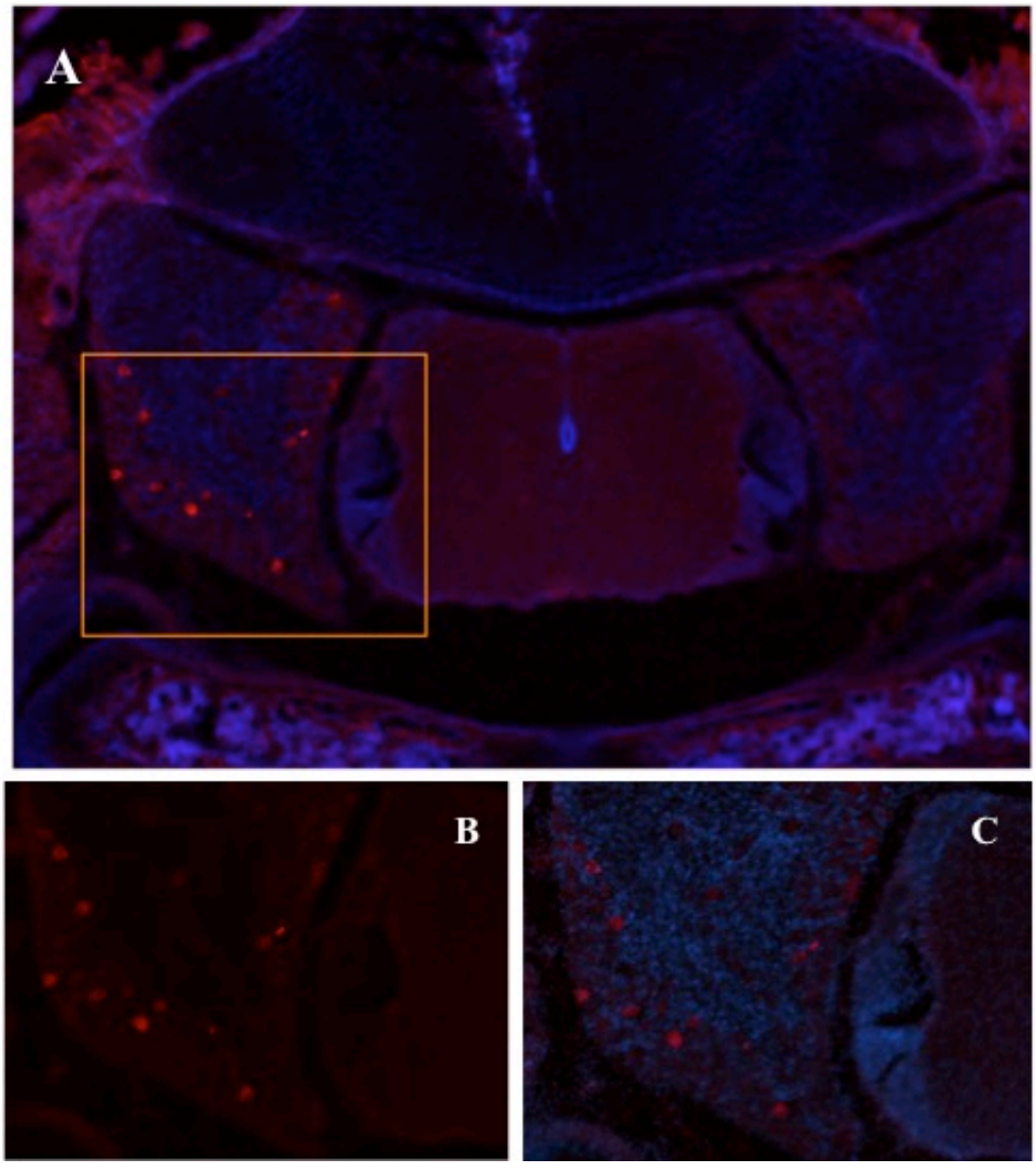

Figure 11: Mice injected with CTB-555 at P1 and collected at P11 showed labeling of DRG, indicating the dye persists for 10 days. Cross section of L5 with CTB-555 (red) co-stained with DAPI (blue) at 4x (a). Inset of panel A shown without DAPI (b) and with DAPI (c).

We then injected neonatal jerboas to identify neuronal cell bodies within two days of birth. We were able to identify motor neurons that were labeled at post-natal day two (P2) (Figure 10 a and b).

In the future, we aspire to retrograde label jerboas for a period of 10 days and then examine the spinal cords to observe if the neuronal cell bodies persist or if they are eliminated.

Merely one week after birth the jerboa foot is completely devoid of muscle, despite the correct initial patterning and differentiation of fetal skeletal foot muscle. My research specifically focused on constructing a timeline for muscle loss and examining the nerve at the neuromuscular junction to assess whether or not the nerve properly innervated the muscle. The data suggest that the foot muscle received proper innervation from the nerve as indicated most strongly by the refinement of Acetylcholine clusters along the nerve path. Furthermore, the synapses appear to begin to disassemble at P4, a stage at which myofibers have also already begun to disassemble and lose their organization. The successful retrograde labeling of motor neurons to their origin in the spinal cord provides further evidence that the neuronal axons remain intact. Also, the process of muscle atrophy due to denervation is known to take months to years to truly develop in adults (B. Carlson, 2014). Considering the muscle begins to disorganize only a few days after birth and is completely absent after one week, it seems unlikely that a lack of innervation in the jerboa foot could cause the muscle to degenerate in such a short period of time.

There are alternate hypotheses as to what could be contributing to muscle loss in the jerboa foot. Mai Tran, a PhD candidate in the Cooper Lab, is actively

investigating other possible mechanisms and has so far found no suggestion of cell death or significant recruitment of macrophages to the foot muscle. The data so far suggests that cell death is unlikely to be occurring. Dr. Rio Tsutsumi, a post doc in the Cooper Lab, is investigating the possibility that myoblasts could be trans-differentiating into tenocytes. Trans-determination of muscle is not known to occur in mammals, therefore the implications of this possible result would provide evidence regarding the plasticity of early muscle fate.

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