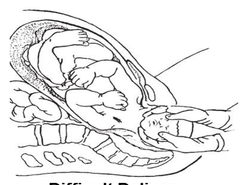
Golgi Tendon Organ Development and Contracture Formation Following Neonatal Brachial Plexus Injury (NBPI)

Tanner Lilly, Sia Nikolaou, Liangjun Hu, Roger Cornwall

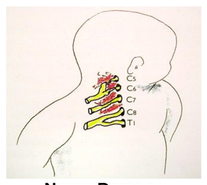
Introduction

The brachial plexus
- A network of nerves originating from the lower cervical (C5-C8) and first thoracic (T1) region of the spinal cord.
- Responsible for the innervation of the upper extremity

Neonatal Brachial Plexus Injury (NBPI)
- A birth injury that involves one or more of the nerves in the brachial plexus.[1]
- NBPI has an incidence of approximately 1.5 per 1000 live births[1] and results in permanent deficits in 20-30% of these cases.[2]



Difficult Delivery



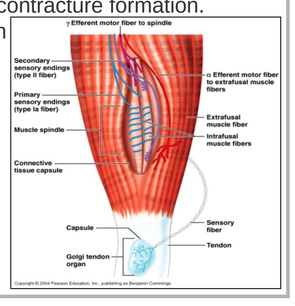
Nerve Damage
(1.5 per 1000 live births)

Joint Contracture
(20-30% persistent defects)

Secondary contracture
- Loss of joint mobility found to form by two weeks post-injury
- Common secondary injury following NBPI
- Shown to result from a denervation induced impairment of muscle growth.
- Preganglionic injuries do not present phenotype[3]
- Pathophysiology is incompletely understood

Afferent innervation in contracture formation
- Afferent innervation is the circuitry that brings information from the periphery to the brain or spinal cord.[4]
- Previous studies have shown preservation of afferent innervation protects against contracture formation.
- Afferent nerves interface with muscle at the muscle spindle and Golgi tendon organ
- Previous studies implicate muscle spindles in contracture formation

Golgi Tendon Organ (GTO)
- Responsible for sensing the stretch in muscle
- Located at the connection between muscle and tendon [5]



Purpose

To better understand the role of afferent (sensory) innervation in GTO maintenance and contracture pathophysiology through developmental analysis of the GTO in the NBPI mouse model.

Hypothesis

We hypothesize that the GTO will be preserved in preganglionic NBPI as a result of the preservation of afferent innervation, whereas following postganglionic NBPI, the GTO will degenerate consistent with complete denervation.

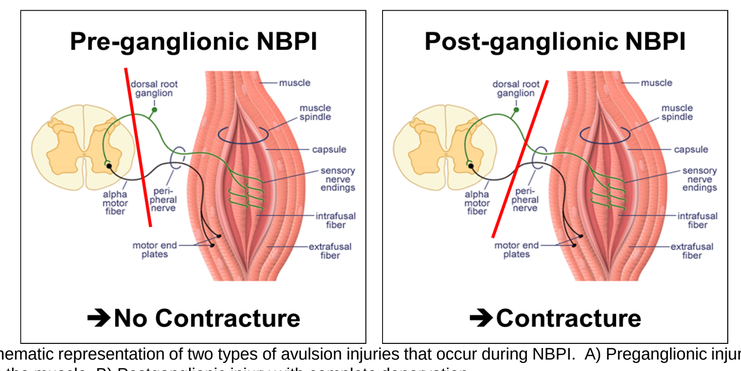


Figure 1. Schematic representation of two types of avulsion injuries that occur during NBPI. A) Preganglionic injury preserving the afferent innervation to the muscle. B) Postganglionic injury with complete denervation

Methods

NBPI Mouse Model
- Created in 5 day old CD1 mice
- Unilateral (upper) C5-C7 pre-ganglionic and post-ganglionic nerve root excision
- Unilateral (global) C5-T1 post-ganglionic nerve root excision

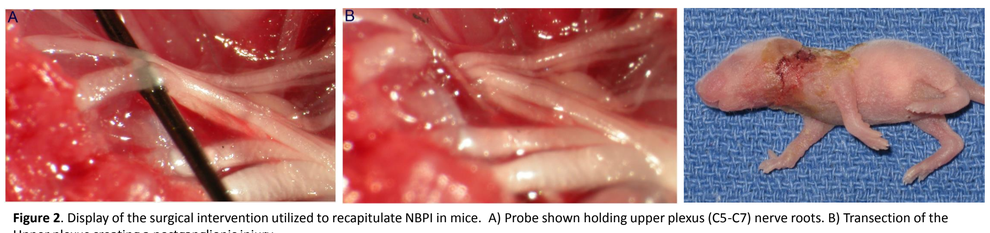
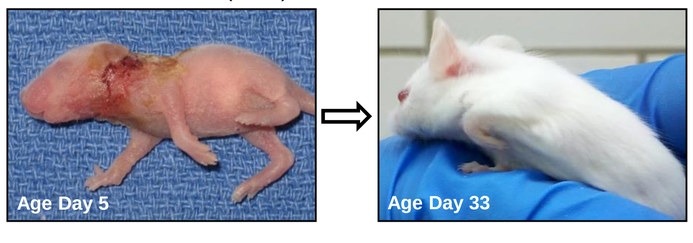


Figure 2. Display of the surgical intervention utilized to recapitulate NBPI in mice. A) Probe shown holding upper plexus (C5-C7) nerve roots. B) Transection of the Upper plexus creating a postganglionic injury

Harvest
- Sacrificed at age day 33
- Limb movement and motor function assessed prior to sacrifice to determine success of surgery
- Biceps and musculocutaneous nerve (MCN) harvested



Processing
- MCN sectioned and stained via standard Immunohistochemistry (IHC) for quantification of afferent denervation
- Biceps cleared using Passive Clarity Technique (PACT)
- Cleared samples stained by Immunohistochemistry (IHC)
- Samples imaged on Nikon A1 LUNA Inverted Confocal Microscope
- Images analyzed via FIJI/NIS Elements software

Results

Elbow flexion contractures occur 4 weeks post-op

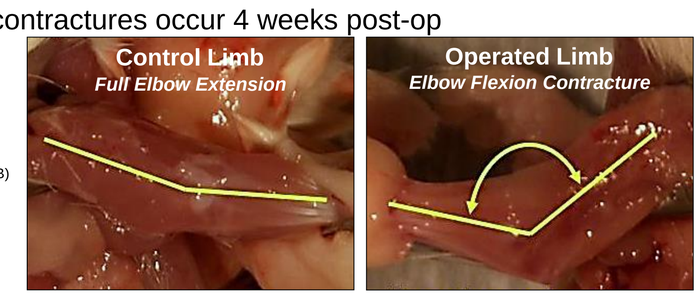


Figure 3. Contracture severity assessed immediately following Sacrifice to determine success of surgery A) Unoperated control limb B) Postganglionic operated limb

GTO morphology was determined to be a single axon or branching of a single axon at the myotendinous junction in the “Goose Quill” region of the distal biceps.

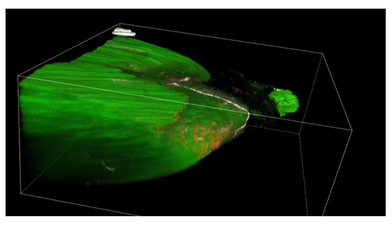
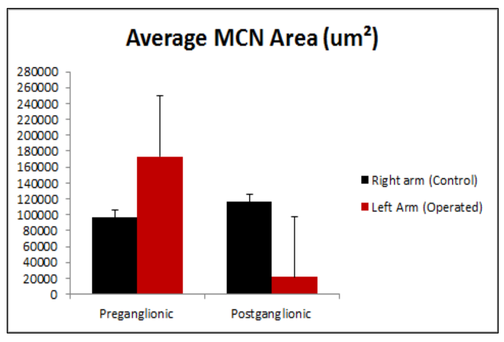


Figure 4. Confocal image of the morphology of the GTO examined by PACT and IHC staining of NF-H axonal marker (Cy5/white), muscle marker Dystrophin (GFP) and tendon marker Tenascin-C (RFP) in the unoperated arm to determine structure and location of distal biceps GTO. Axon

Musculocutaneous nerve (MCN) degenerated following postganglionic NBPI



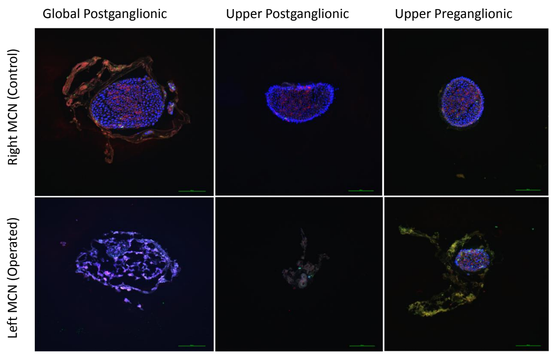


Figure 5. Musculocutaneous nerve sections stained with Neurofilament-H (green), Tyrosine Hydroxylase (red) and Parvalbumin (blue) in order to quantify the effects of both types of injury. Total cross sectional area of each sample used for a relative size measurement.

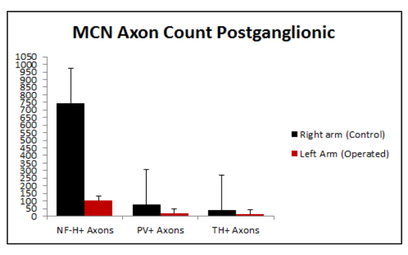
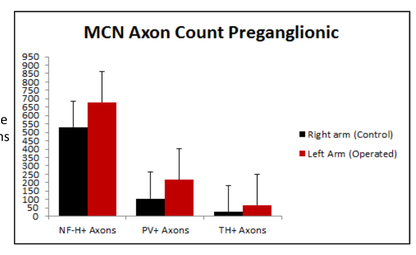


Figure 6. Quantification of the axonal contents in the MCN following pre and postganglionic NBPI show a decrease in the number of NF-H⁺ axons as well as PV⁺ axons following postganglionic injury.



The GTO degenerated following both upper (C5-7) and global (C5-T1) postganglionic NBPI. Normal GTO morphology was preserved following preganglionic NBPI.

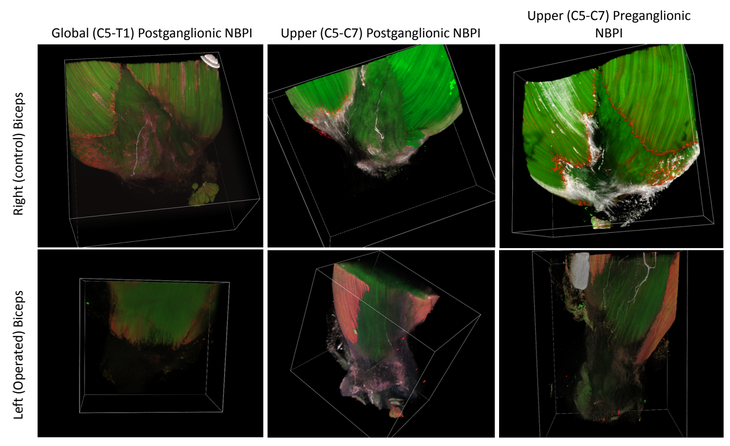


Figure 7. Confocal images of whole mount biceps muscle samples cleared via PACT and Immunohistochemically stained with axonal marker Neurofilament-H (Cy5/ White), muscle marker dystrophin (GFP), and tendon marker tenascin-c (RFP). Upper preganglionic injury displays preservation of the GTO following denervation injury. Both upper and global postganglionic samples displayed a degeneration in GTO.

Conclusion

- Complete denervation through postganglionic NBPI caused a complete loss of the Golgi tendon organ in the distal biceps.
- Preservation of afferent innervation as occurs in the less common preganglionic NBPI preserved Golgi tendon organ morphology.
- These findings suggest afferent innervation is required for postnatal GTO maintenance.
- Loss of the GTO may play a role in contracture formation
- Future directions include:
  - Further analysis of the Golgi tendon organ in the biceps following NBPI
  - Analysis of the relationship between the GTO and muscle growth
  - Repeat experiment with the use of a transgenic mouse line with a proprioceptive reporter to obtain better images

References

1) Foad, S.L., C.T. Mehlman, and J. Ying, The epidemiology of neonatal brachial plexus palsy in the United States. J Bone Joint Surg Am, 2008. 90(6):1258-64.
2) Pondaag, W., et al., Natural history of obstetric brachial plexus palsy: a systematic review. Dev Med Child Neurol, 2004. 46(2):138-44.
3) Al-Qattan, M.M., Obstetric brachial plexus palsy associated with breech delivery. Ann Plast Surg, 2003. 51(3):257-64; discussion 265.
4) Purves, D., et al., Neuroscience., 5th edition. Sinauer Associates, Inc, 2012. 1,9:11,187-207.
5) Sonner MJ, Walters MC, Ladle DR (2017) Analysis of Proprioceptive Sensory Innervation of the Mouse Soleus: A Whole-Mount Muscle Approach. PLoS ONE 12(1): e0170751.