Adhesions and Paraneurial Tissues Create Strain Gradients In Rat Sciatic Nerves

Ian Foran M.D., Vince Hussey B.S., Rushil Patel, Jaemyoung Sung, Sameer Shah Ph.D.
University of California, San Diego School of Medicine
Department of Orthopaedic Surgery

Introduction/Background:

- Although nerve entrapment syndromes like carpal tunnel syndrome are well-described disease entities, their etiologies are unclear.
- The role that adhesions and paraneurial soft tissues play in entrapment syndromes is unknown
- Surgical decompression is performed to release nerves from soft tissue tethers. It is unknown how this affects regional nerve strain.

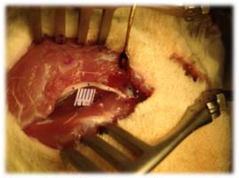
Aims:

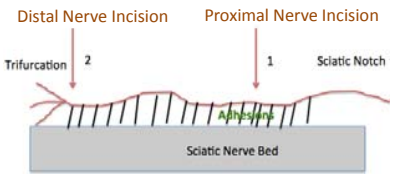
1. Investigate the impact of adhesions and paraneurium on regional nerve strain.
2. Study the effect of surgical decompression of these tissues on nerve strain.

Methods:

Rat Surgery and Strain Measurements:

1. Rat sacrificed according to IACUC protocol.
2. Sciatic nerve exposed.
3. 5 marks made on sciatic epineurium 3-5mm apart with tissue marker.
4. Knee extended and ankle dorsiflexed; strain measurements taken
5. Soft tissue connections incised with scissors; strain measurements repeated.
6. After six weeks of monitoring, the sciatic nerve was again exposed, and strains measured as above before and after decompression of newly formed adhesions.
6. Nerve carefully harvested and frozen down for future sectioning.





Tissue Sectioning and Immunohistochemistry:

1. Nerves embedded in OCT and flash frozen in liquid nitrogen cooled isopentane.
2. Tissue sectioned with cryostat (Leica CM 3050 S) into 10-µm-thick cross sections.
3. Sections fixed with 10% Formalin, permeated with 0.2% Triton X-100 and blocked with 10% normal goat serum and 3% BSA in PBS.
4. Sections incubated with primary antibodies to laminin (1:500, Sigma-Aldrich) and neurofilament (1:1000, Sigma-Aldrich) for 1 hour at RT.
5. Sections incubated with Alexa Fluor-405 goat anti-mouse (1:200, Life Technologies) and Alexa Fluor-488 goat anti-rabbit (1:200, Life Technologies) for 1 hour at RT.

Trichrome Staining:

1. Neutral Buffered Formalin (10%) for 30 minutes.
2. Filtered Harris's Haematoxylin for 4 minutes.
3. Gömöri's Trichrome Stain (Fast Green FCF, Phosphotungstic acid, Chromotrope 2R.
4. Standard Staining Protocol.

Trichrome Scoring:

1. Blinded images were presented to 5 individuals.
2. Scored based on a scale from 1 (full adhesion formation) to 5 (no adhesion formation).
3. Sample images for each score were presented to individuals as training, prior to testing the full set of images
4. Average scores for each image were determined and a one-way analysis of variance (ANOVA) was used to test for significance between groups.

Results:

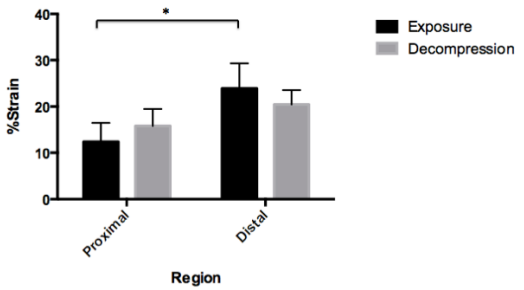


Fig 1. Average sciatic nerve strain before and after decompression of normal paraneurial tissues on Day 0. Note that normal strain gradient is lost after paraneurial tissues are decompressed ($p<0.05$).

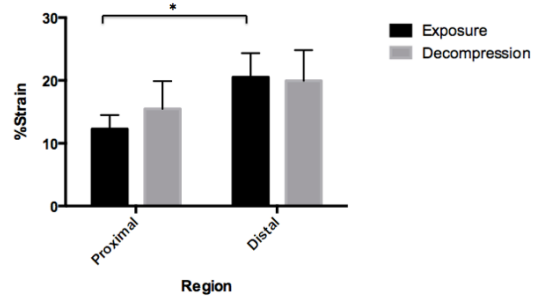


Fig 2. Average sciatic nerve strain before and after decompression of **adhesions** on Day 42. Note that original strain gradient seen on Day 0 is again present prior to decompression, and then lost after adhesions are decompressed ($p<0.05$).

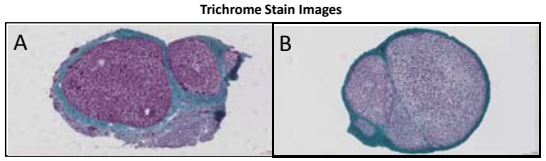


Fig. 5. Representative images of Trichrome stained rat sciatic nerve cross-sections. (A) >50% adhesion formation, score of 1. (B) No adhesion formation, score of 5.

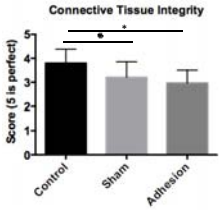


Fig. 6. Quantitative analysis of adhesion formation between groups. * indicates significant difference between average scores, p<.001.

Fig. 7. Representative immunoflourescent images of rat sciatic nerve cross-sections from adhesion (A), sham (B), and control (C) groups. Laminin labeled in green, neurofilament labeled in red. No significant difference in axon counts or laminin density observed.

Conclusion:

- Prior to decompression, strains are more highly concentrated in the distal nerve region than the proximal nerve region.
- Formation of adhesions recreates the strain gradient from proximal to distal seen with normal paraneurial tissues after 6 weeks
- Releasing paraneurium **or** adhesions results in homogenization of strain across the measured region of nerve.
- Adhiesons have no effect on axon quantity or laminin density.
- **Adhesions may represent a more organized form of healing with respect to nerves than previously surmised.**

Limitations:

- The measured region was not located at a joint, where nerves are known to elongate the most.
- Epineurial, rather than true intraneural strain, was measured.
- Outcomes were biomechanical only. We did not analyze function in this study (eg. EMG, walking track analysis).

Extension:

- We are currently investigating the effect of immobilization on nerve strain.
- We are currently analyzing the effect of adhesions of nerve function
- We are investing possible compounds that may prevent adhesions, and the effect that these may have on nerves strain gradients and function

Acknowledgements:

We gratefully acknowledge Sam Ward PhD, Matt Love PhD, Brian Bober PhD, and members of the Neuromuscular Bioengineering Laboratory for the support and participating in discussions about this project.

References

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