UNIVERSITY OF SOUTH CAROLINA School of Medicine

VANDERBILT UNIVERSITY MEDICAL CENTER

Assessment of the Effect of Autograft Orientation on Peripheral Nerve Regeneration Utilizing Diffusion Tensor Imaging

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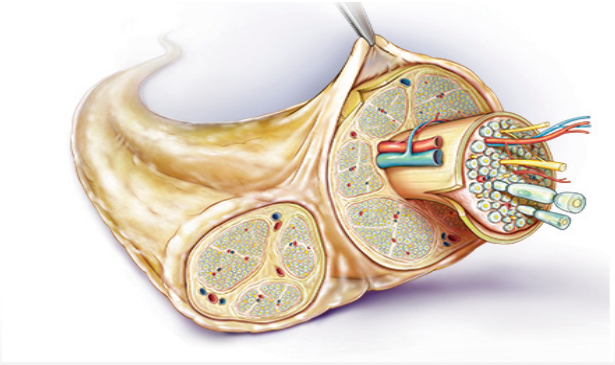


Disclosure :The authors have nothing to disclose.

Nerve Autograft

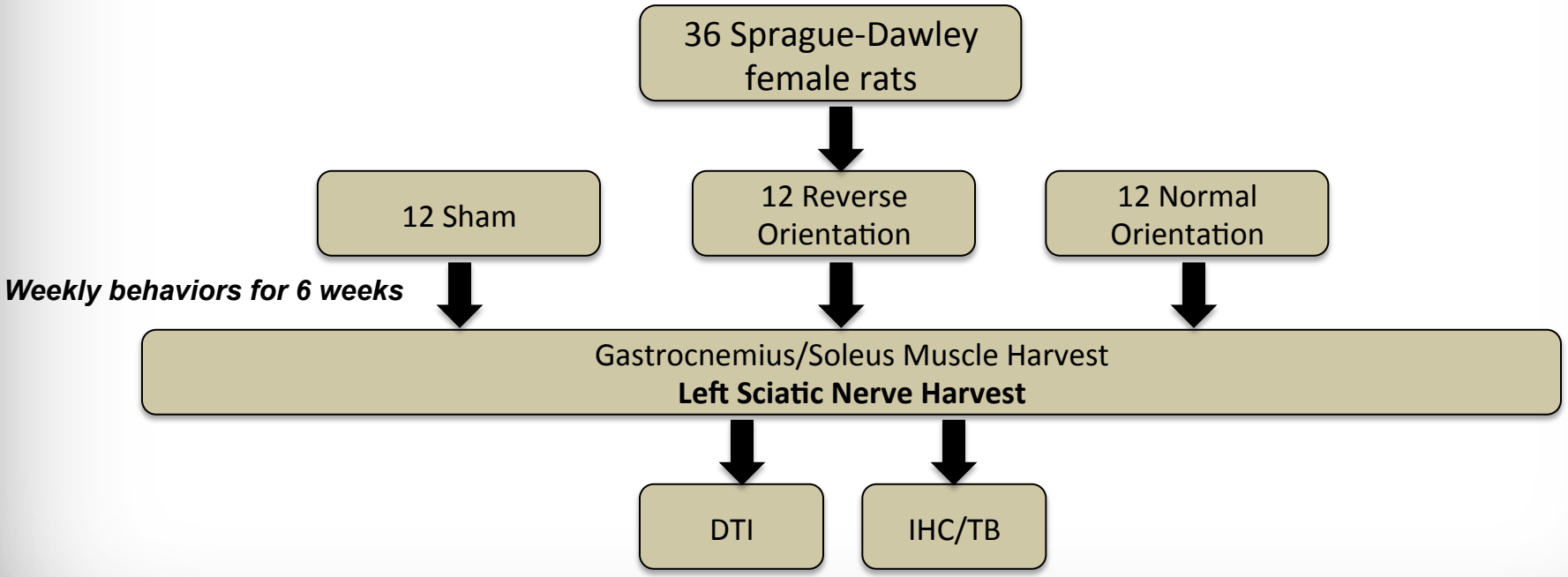
- Few studies to evaluate the effect of autograft polarity.
  - Limited by number of assessment tools.
- No consensus on role of autograft orientation.

Stromberg B V, Vlastou C, Earle A S. Effect of nerve graft polarity on nerve regeneration and function. *J Hand Surg*. 1979;4:444-5.
Sotereanos DG, Seaber AV, Urbaniak JR. Reversing nerve-graft polarity in a rat model: the effect on function. *J Reconstr Microsurg*. 1992;8:303-7.
Nakatsuka H, Takamatsu K, Koshimune M, et al. Experimental study of polarity in reversing cable nerve grafts. *J Recontr Microsurg*. 2002;18(6):509-15.
Ansselin AD, Davey DF. The regeneration of axons through normal and reversed peripheral nerve grafts. *Restor Neurol Neurosci*. 1993;5(3):225-40.

Purpose

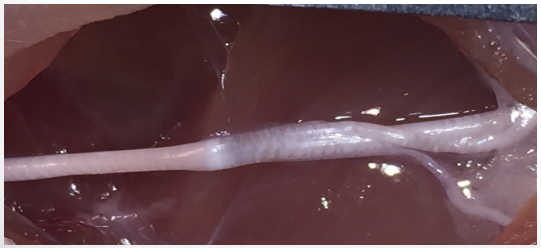
- Evaluate the effect of autograft orientation on nerve recovery using multiple assessments tools, including DTI.

Methods (Design)

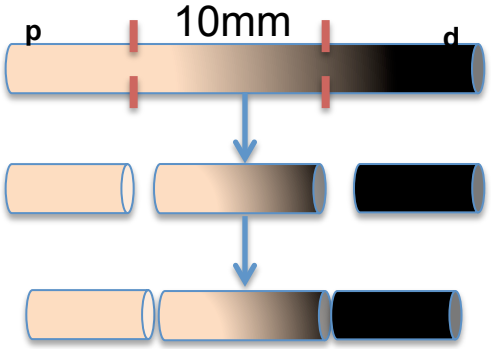


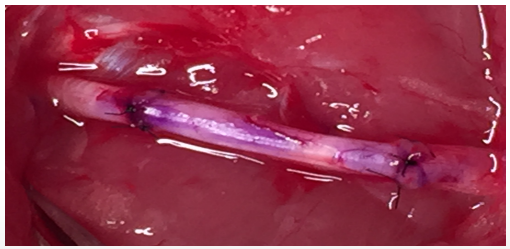
Methods (Microsurgery)

Sham

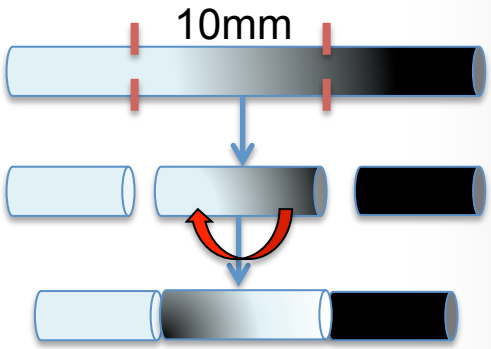


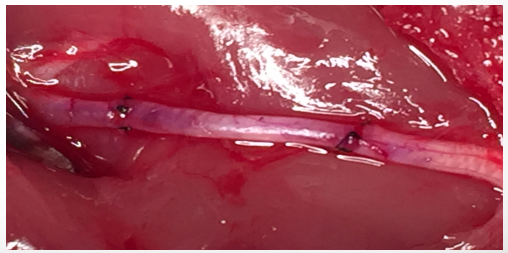
Normal Orientation





Reverse Orientation

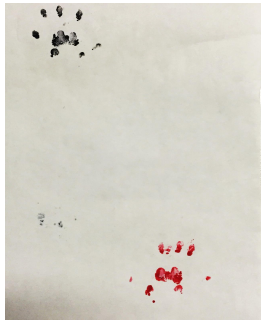


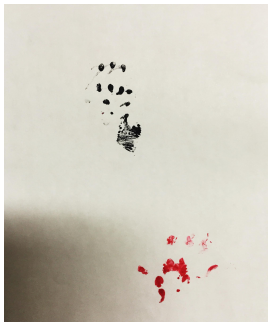


Behavior: Sciatic Function Index

- Hind limbs inked and animal walks up an inclined plank

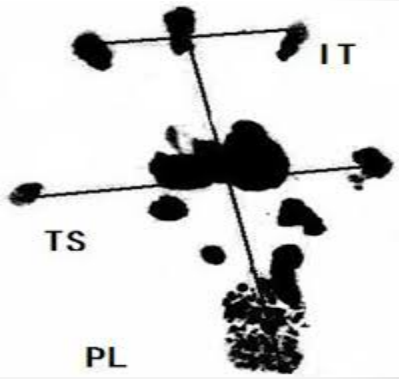
- Markings measured and inserted into a validated formula





- Greater impairment demonstrated by more negative score

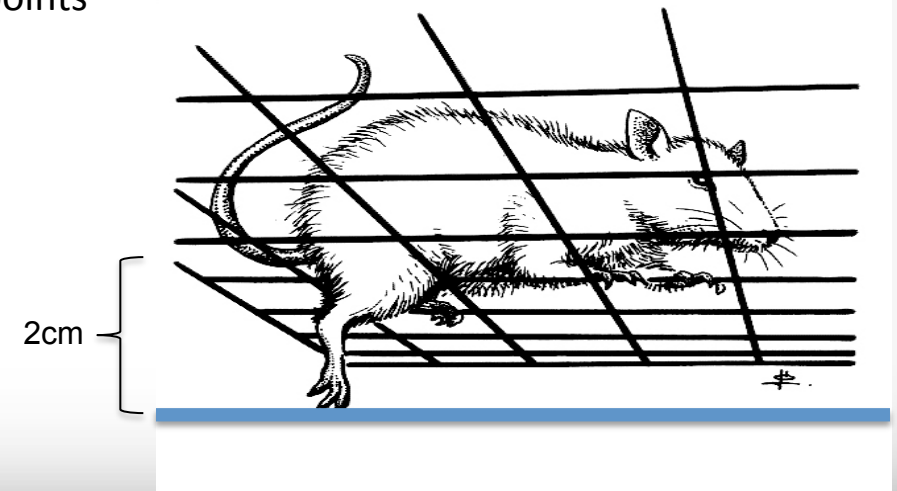
$$SFI = -38.3\times\left(\frac{EPL-NPL}{NPL}\right)+109.5\times\left(\frac{ETS-NTS}{NTS}\right)+13.3\times\left(\frac{EIT-NIT}{NIT}\right)-8.8$$



Behavior: Foot Fault

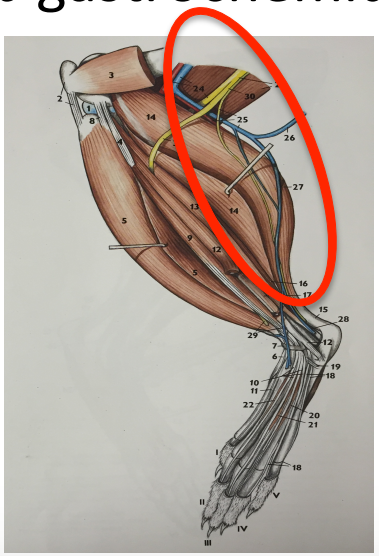
- Animals allowed to take 50 steps/hind limb on wired grid, and number of foot faults (FF) recorded
  - Partial FF (through grid without touching base) = 1 point
  - Full FF (through grid and touches base)= 2 points

- **Foot Fault Asymmetry Score**= %foot fault (surgical hind limb) - %foot fault (normal hind limb)



Muscle Net Weight

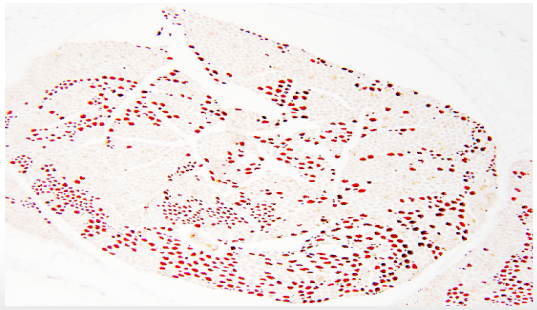
- Net weight (gm)= weight (normal limb gastrocnemius/soleus m.)- weight (surgical limb gastrocnemius/soleus m.)



Histology

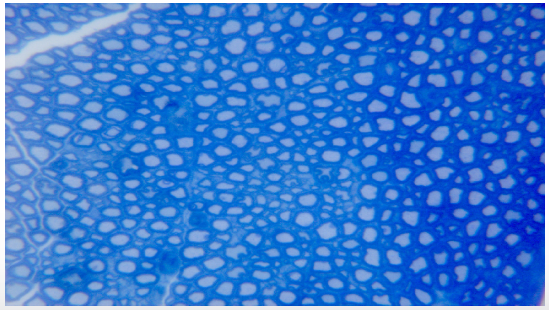
Immunohistochemistry

- 5 µm thick, Choline-acetyltransferase (ChAT) stained for motor axon counts at 10X



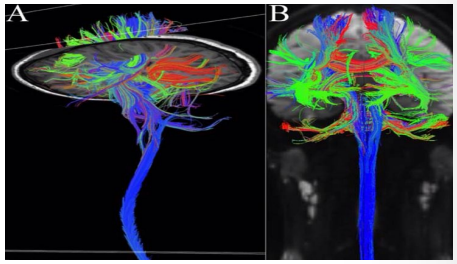
Toluidine Blue

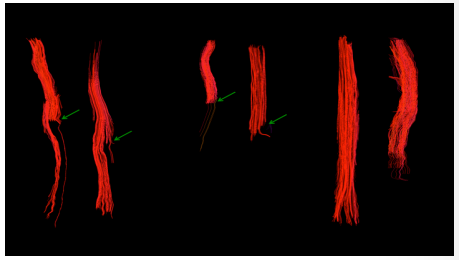
- 1 µm thick sections
- Axon count, density and diameter at 40X

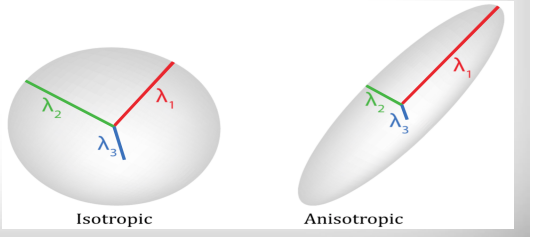


Diffusion Tensor Imaging (DTI)

- Common tool used in evaluation of CNS; emerging MRI technique for PNS.
- Relies on diffusion of water molecules within tissue.
- Fractional anisotropy, axial and radial diffusivity, and tractography data obtained.

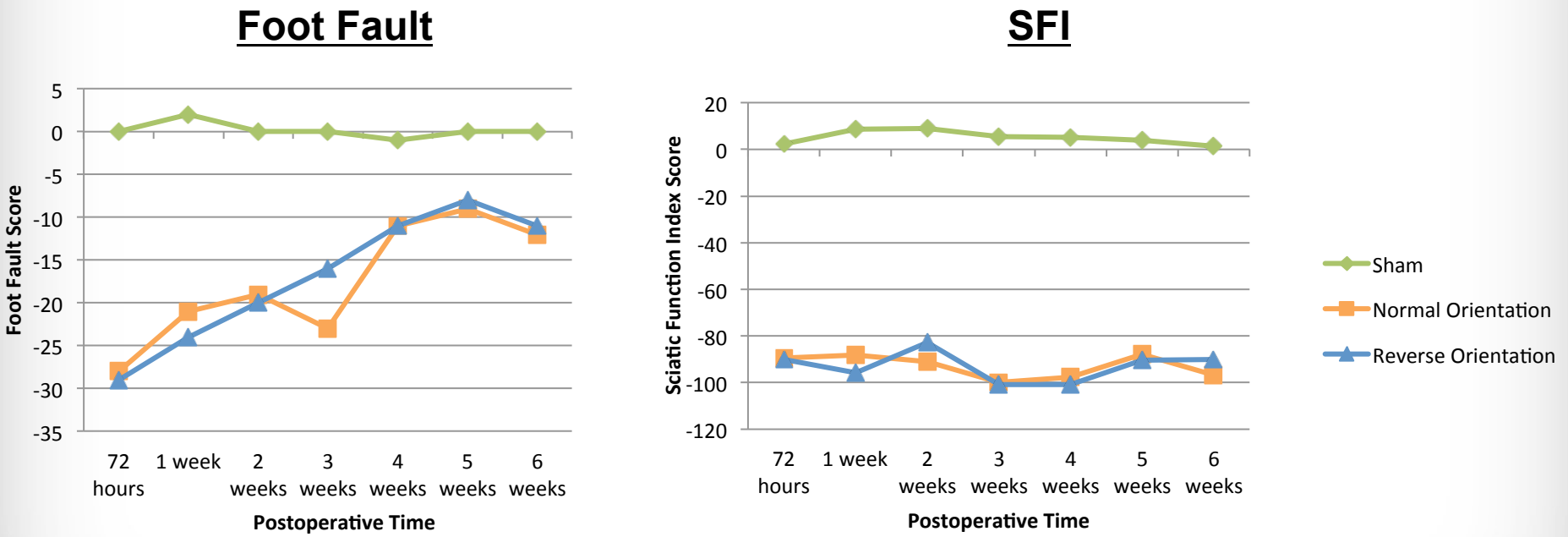






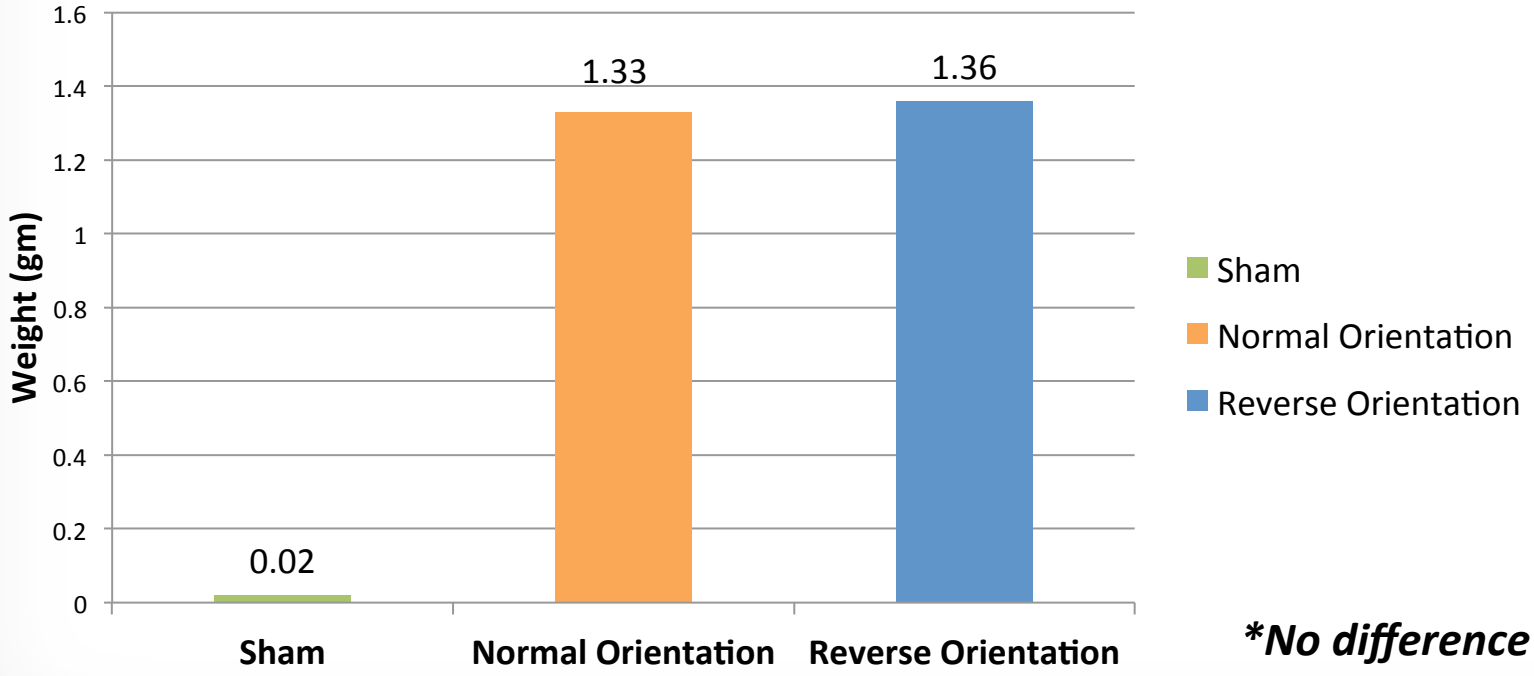
Lehmann HC, Zhang J, Mori S, Sheikh KA. Diffusion tensor imaging to assess axonal regeneration in peripheral nerves. *Exp Neurol*. 2010;223(1):238-44.
Sheikh KA. Non-invasive imaging of nerve regeneration. *Exp Neurol*. 2010;223(1):72-6.

Results: Behavior studies



No difference in FF or SFI between normal and reverse autografts

Results: Muscle Net Weight

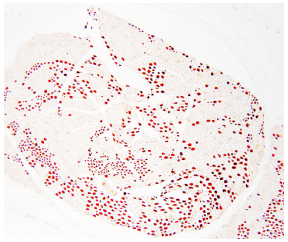


No difference between autograft groups

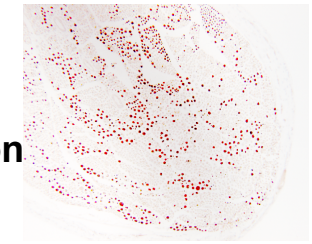
Results: Motor IHC

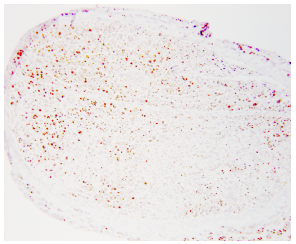
Proximal	Graft	Distal

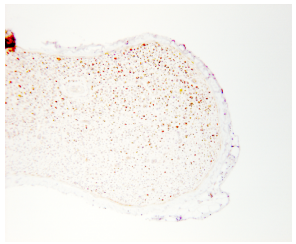
Sham



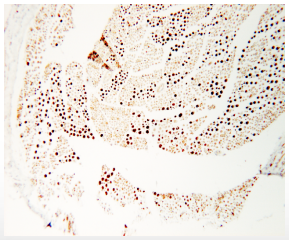
Normal Orientation

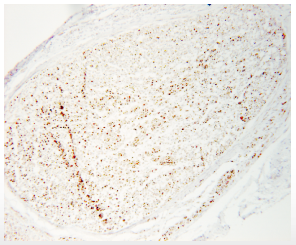


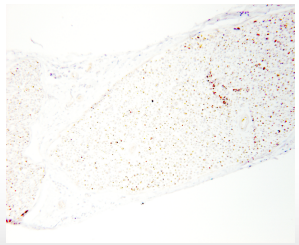


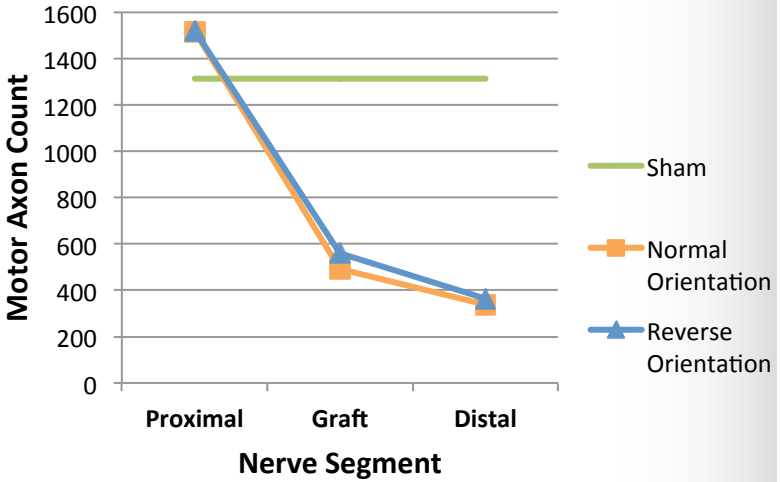


Reverse Orientation



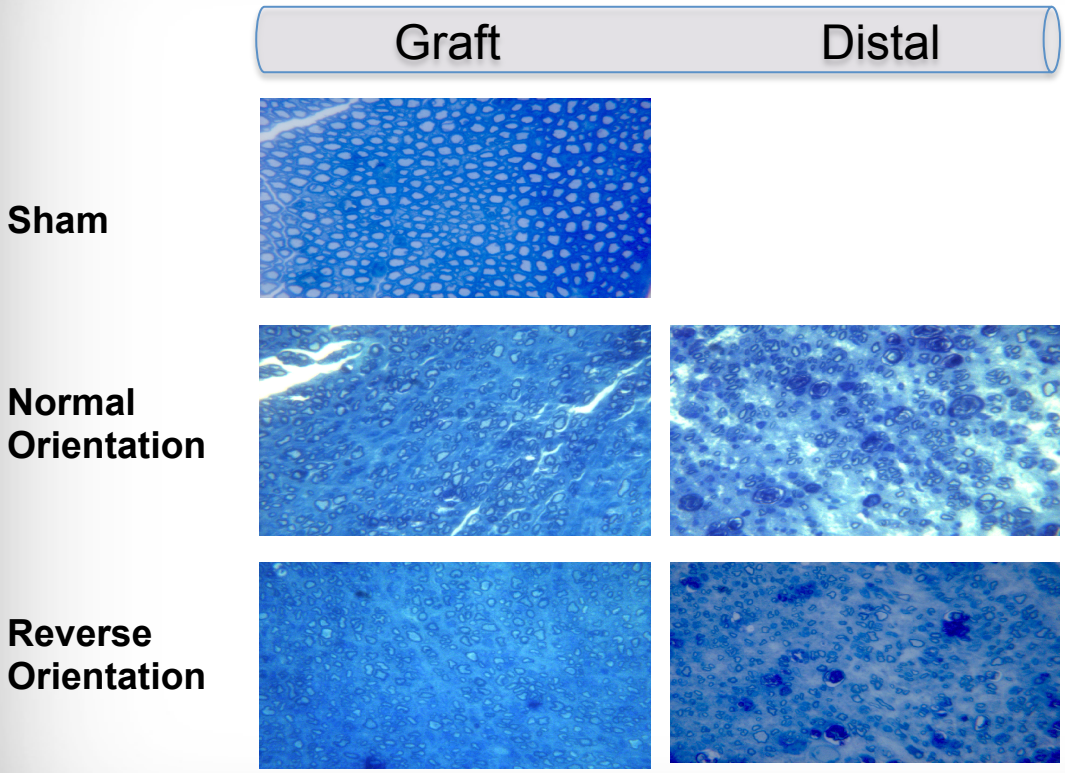


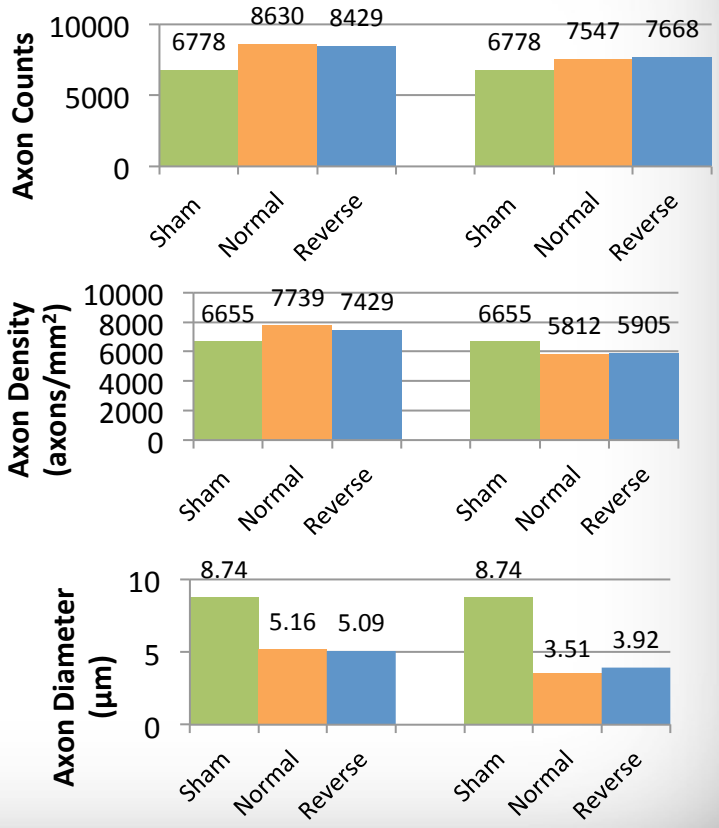




No difference in motor axon count between normal and reverse autografts at any nerve segment

Results: Toluidine Blue





No difference in axon count, density or diameter between normal and reverse autografts within and distal to the autograft

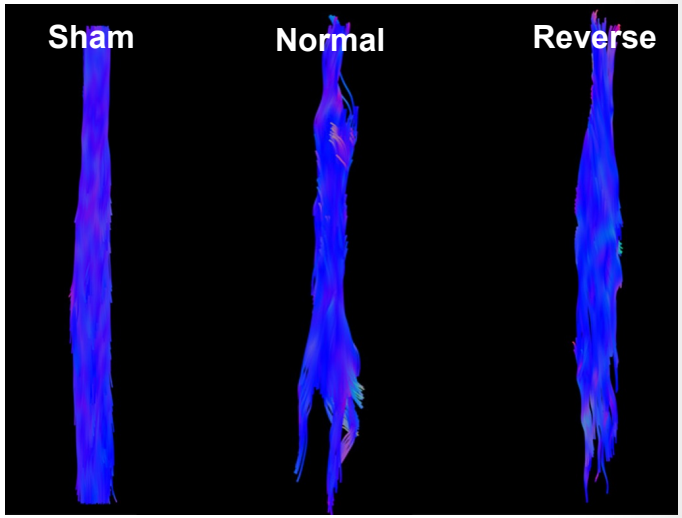
Results: DTI

Comparison of DTI parameters between normal and reverse autografts at all nerve segments

	Proximal	IQR	*p*	Graft	IQR	*p*	Distal	IQR	*p*
Fractional Anisotropy (FA)									
Sham	0.70	0.68, 0.72							
Normal Orientation	0.55	0.49, 0.57	0.57	0.59	0.50, 0.64	0.57	0.56	0.48, 0.61	1.00
Reverse Orientation	0.53	0.49, 0.56		0.55	0.53, 0.60		0.54	0.51, 0.58	
Axial Diffusivity (AD) ($\mu m^2/ms$)									
Sham	0.82	0.80, 0.85							
Normal Orientation	0.82	0.78, 0.83	1.00	0.78	0.69, 0.79	0.57	0.77	0.75, 0.78	1.00
Reverse Orientation	0.81	0.76, 0.87		0.76	0.75, 0.83		0.77	0.76, 0.82	
Radial diffusivity (RD) ($\mu m^2/ms$)									
Sham	0.24	0.22, 0.25							
Normal Orientation	0.34	0.34, 0.37	0.57	0.32	0.29, 0.34	0.57	0.34	0.32, 0.37	1.00
Reverse Orientation	0.38	0.34, 0.44		0.34	0.31, 0.39		0.35	0.33, 0.40	

IQR: interquartile range; Statistical significance, $p<0.05$.

No difference in FA, AD, RD between normal and reverse autografts at any nerve segment



Proximo-distal axonal growth demonstrated in normal and reverse autografts

Conclusion

- Nerve regeneration was similar in reverse- and normal-oriented autografts.
- Autograft polarity may not influence nerve regenerative outcomes.
- Nerve repairs utilizing non-branched autografts should be performed using principles (i.e. best fascicular alignment) other than orientation to maximize regeneration.