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.

Purpose

• Evaluate the effect of autograft orientation on nerve recovery using multiple assessments tools, including DTI.
Methods (Design)

- 36 Sprague-Dawley female rats
  - 12 Sham
  - 12 Reverse Orientation
  - 12 Normal Orientation

Gastrocnemius/Soleus Muscle Harvest
Left Sciatic Nerve Harvest

Weekly behaviors for 6 weeks

- DTI
- IHC/TB
Methods (Microsurgery)

- Sham
- Normal Orientation: 10mm
- Reverse Orientation: 10mm
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.


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

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Nerve Segment</th>
<th>Motor Axon Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Proximal</td>
<td>1600</td>
</tr>
<tr>
<td></td>
<td>Graft</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>1200</td>
</tr>
<tr>
<td>Reverse</td>
<td>Proximal</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Graft</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>400</td>
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</tbody>
</table>

*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

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>IQR</th>
<th>p</th>
<th>Graft</th>
<th>IQR</th>
<th>p</th>
<th>Distal</th>
<th>IQR</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Fractional Anisotropy (FA)</strong></td>
<td></td>
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<tr>
<td>Sham</td>
<td>0.70</td>
<td>0.68, 0.72</td>
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<tr>
<td>Normal Orientation</td>
<td>0.55</td>
<td>0.49, 0.57</td>
<td>0.57</td>
<td>0.59</td>
<td>0.50, 0.64</td>
<td>0.57</td>
<td>0.56</td>
<td>0.48, 0.61</td>
<td>1.00</td>
</tr>
<tr>
<td>Reverse Orientation</td>
<td>0.53</td>
<td>0.49, 0.56</td>
<td>0.55</td>
<td>0.55</td>
<td>0.53, 0.60</td>
<td>0.54</td>
<td>0.51</td>
<td>0.58</td>
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<tr>
<td><strong>Axial Diffusivity (AD)</strong> (μm²/ms)</td>
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<tr>
<td>Sham</td>
<td>0.82</td>
<td>0.80, 0.85</td>
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<td>Normal Orientation</td>
<td>0.82</td>
<td>0.78, 0.83</td>
<td>1.00</td>
<td>0.78</td>
<td>0.69, 0.79</td>
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<td>Reverse Orientation</td>
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<td>0.76, 0.87</td>
<td>0.76</td>
<td>0.76</td>
<td>0.75, 0.83</td>
<td>0.77</td>
<td>0.76</td>
<td>0.82</td>
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<tr>
<td><strong>Radial diffusivity (RD)</strong> (μm²/ms)</td>
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<tr>
<td>Sham</td>
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<td>0.22, 0.25</td>
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<tr>
<td>Normal Orientation</td>
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<td>0.34, 0.37</td>
<td>0.57</td>
<td>0.32</td>
<td>0.29, 0.34</td>
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<td>0.32, 0.37</td>
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<tr>
<td>Reverse Orientation</td>
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<td>0.34</td>
<td>0.34</td>
<td>0.31, 0.39</td>
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<td>0.33</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

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

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