Chronically Denervated Versus Fresh Nerve Graft

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Introduction:
Nerve grafting is a well-established strategy for reconstructing nerve defects. Donor nerve selection may affect outcome (3). Several clinical scenarios (such as brachial plexus and sciatic nerve injuries) encourage the use of nerve graft material that has been denervated for varying lengths of time. There is data that suggests that this additional denervation period may be detrimental to the effectiveness of this tissue as nerve graft material yet this variable has never been independently evaluated (1, 2). Our hypothesis is that the longer a nerve graft is denervated the more detrimental impact this will have on functional outcome.

Materials/Methods:
Fifty-five immature female Sprague-Dawley rats were used in this study. All experimental procedures and care of animals was approved by our institutions IACUC review board and according to nationally accepted guidelines. The rats were divided into four groups: A, B, C, Sham with an n=15 for each group except the Sham group with an n=10. Forty-five rats (groups A,B,C) underwent transection of the right peroneal nerve at the sciatic nerve bifurcation using the standard biceps femoris semi-tendinosus muscle splitting approach. The Sham group underwent exposure of said nerve without manipulation. After two months (B group, n=15) or four months (C group, n=15), these rats underwent removal of one centimeter of the contralateral peroneal nerve with grafting of the subsequent defect with one centimeter of the previously transected (denervated) peroneal nerve. A control group (A group, n=15), underwent removal of one centimeter of the contralateral peroneal nerve with immediate grafting of the subsequent defect with the excised segment (fresh graft). Each of these three groups (A,B,C) were given 8 weeks of regeneration time before testing. The Sham group underwent testing at the same time as the experimental groups. Testing consisted of stimulation of the proximal sciatic nerve and twitch contraction force measurements from the extensor digitorum longus (EDL). This muscle was then measured and weighed. The repaired peroneal nerves (and one peroneal nerve from the Sham rats) were harvested and stored in 10% formalin for toluidine blue staining and histological analysis including axonal counts and g-ratios. Means were calculated and reported with ±SEM and were compared between groups head-to-head for statistical significance using unpaired Student’s t-test and one-way ANOVA for testing across multiple groups with a p<0.05 considered significant.

Results:
Mean muscle contraction forces for the reinnervated EDL were 58% of the normal rat for the rats repaired with fresh nerve graft, 57% for the rats repaired with two month old denervated nerve graft, and 64% for the rats repaired with four month old denervated nerve graft. There were no statistical differences between the contraction forces of any of the experimental groups though all were less than the sham group (p<0.05)(Fig 1).

Conclusion:
Prolonged denervation of nerve graft material of up to four months did not inhibit reinnervated muscle contraction strength though muscle size and weight were both diminished. Axons were decreased in number but increased in size in the prolonged denervated group(p<.05). This suggests that less robust reinnervation was seen in the prolonged denervated graft group. While the use of rats as a model for nerve studies is widely accepted it introduces a degree of error when attempting to apply these findings to clinical scenarios. Further investigations into improved nerve regeneration techniques following use of nerve graft are appropriate.

References:

Figure 1. There was no statistical difference across the experimental groups in muscle strength of contraction. All three groups showed a statistically significant decrease in muscle contraction compared to the sham group (p<0.05).

Figure 2. The number of axons decreased with the longer denervation time (p<0.05).

Figure 3. There was no statistical difference in the (g-ratio) between the three experimental groups. There was a statistical difference between the experimental groups and the Sham group (p<0.05).

Myelination (as measured by G-ratio=axon diameter/total fiber diameter) was statistically the same between all three experimental groups (fig 3). Interestingly, the average axonal size increased with longer denervation time with the four month denervated group having the largest axons on average (p<0.05).