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

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Abstract

Purpose—Given no definite consensus on the accepted autograft orientation during peripheral nerve injury repair, we compare outcomes between reverse and normally oriented autografts using an advanced magnetic resonance imaging technique, diffusion tensor imaging.

Methods—Thirty-six female Sprague-Dawley rats were divided into 3 groups: sham—left sciatic nerve isolation without injury, reverse autograft—10-mm cut left sciatic nerve segment reoriented 180° and used to coapt the proximal and distal stumps, or normally oriented autograft—10-mm cut nerve segment kept in its normal orientation for coaptation. Animals underwent sciatic functional index and foot fault behavior studies at 72 hours, and then weekly. At 6 weeks, axons proximal, within, and distal to the autograft were evaluated using diffusion tensor imaging and choline acetyltransferase motor staining for immunohistochemistry. Toluidine blue staining of 1-μm sections was used to assess axon count, density, and diameter. Bilateral gastrocnemius/soleus muscle weights were compared to obtain a net wet weight. Comparison of the groups was performed using Mann-Whitney *U* or Kruskal-Wallis *H* tests to determine significance.

Results—Diffusion tensor imaging findings including fractional anisotropy, radial diffusivity, and axial diffusivity were similar between reverse and normally oriented autografts. Diffusion tensor imaging tractography demonstrated proximodistal nerve regeneration in both autograft groups. Motor axon counts proximal, within, and distal to the autografts were similar. Likewise, axon count, density, and diameter were similar between the autograft groups. Muscle net weight at 6 weeks and behavioral outcomes (sciatic functional index and foot fault) at any tested time point were also similar between reverse and normally oriented autografts.

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Conclusions—Diffusion tensor imaging may be a useful assessment tool for peripheral nerve regeneration. Reversing nerve autograft polarity did not demonstrate to have an influence on functional or regenerative outcomes.

Keywords

autograft; diffusion tensor imaging; orientation; polarity; peripheral nerve regeneration; DTI

Autologous nerve grafting is considered the gold standard to bridge nerve gaps. Autograft orientation, reversed or normal, has previously been studied to determine the effects of polarity on nerve regeneration. In both histological and functional indices, studies have demonstrated no differences found with the orientation of nonbranched autografts.^{1–3} Yet, an animal study by Ansselin and Davey^{4,5} demonstrated a greater cross-sectional area and, perhaps, improved axonal regeneration at the distal stump with reversed autografts with branch points. Among the few published studies on this topic, each uses a limited number of assessment tools to compare outcomes between reverse and normally oriented autografts. Therefore, there is uncertainty with respect to the best nerve autograft orientation during peripheral nerve repair.

Recently, more advanced methods of determining nerve growth have been developed. Diffusion tensor imaging (DTI) is a magnetic resonance imaging method that has the ability to noninvasively assess axonal integrity and regeneration in peripheral nerves objectively and visually.^{6,7} Diffusion tensor imaging parameters such as fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD) reflect the anisotropic diffusion of water molecules along and perpendicular to axon bundles, which are affected by axon density and myelin thickness.⁸ Values obtained from DTI aid in assessing nerve regeneration and degeneration. Furthermore, DTI allows visualization of neural tracts and assessment of changes in axon microstructure.^{6–8} Using this emerging technology in addition to traditional assessment tools, this study provides a comprehensive comparison of reverse and normally oriented autograft repairs in a nonbranched model.

METHODS

Experimental Design

This study was performed using a rat sciatic nerve model using a total of 36 animals divided into 3 groups: reverse autografts (n = 12), normally oriented autografts (n = 12), and shams (n = 12) to serve as a control. Postoperatively, all rats underwent behavioral studies at 72 hours, and then weekly. At 6 weeks, the left sciatic nerve was harvested; half were evaluated using (1) immunohistochemical staining of 5- μ m-thick sections and toluidine blue staining of 1- μ m-thick sections or (2) DTI at segments proximal, within, and distal to the autografts. Only 1 segment was evaluated in the sham group. Bilateral gastrocnemius/soleus muscles were harvested to obtain net wet weights to assess the degree of muscle atrophy.

Animal Model

Adult female Sprague-Dawley rats (200–250 g; Charles River Lab) were used in the experiment. Animals were housed in a central animal care facility and provided food and

water ad libitum. All experimental procedures were approved and performed in accordance with the standards of our institutional animal care and use committee.

Surgical Model

Animals were anesthetized via inhaled isoflurane (2%). The left hindquarter was shaved and prepped in a sterile fashion. A 3-cm incision was made from the left sciatic notch and extended inferiorly, parallel and just posterior to the left femur. The biceps femoris was reflected to expose the sciatic nerve. The nerve was trimmed free of any perineural tissue for all groups. For the autograft groups, a 10-mm nerve gap was created in the mid-portion of the sciatic nerve with microsurgical scissors. The transected portion of the nerve was either coapted to the sciatic nerve in normal orientation or coapted after reversing the cut segment 180° proximodistally. An end-to-end repair using 4 equidistant 9-0 nylon sutures (Ethicon, Somerville, NJ) around each end of the autograft was performed using standard microsurgical technique under a Carl Zeiss Stemi Sv-11 microscope (Thornwood, NY). For all groups, the muscle was imbricated to cover the nerve using 5-0 monocryl in a horizontal mattress fashion. The skin was closed in a running subcuticular fashion with 5-0 monocryl suture.

Behavioral Testing

Before surgery, animals underwent habituation trials for acclimation purposes. Postoperatively, foot fault (FF) asymmetry and sciatic functional index (SFI) test were performed at 72 hours followed by weekly intervals, up to 6 weeks. To prevent possible bias, behaviors testers were blinded to the surgical protocols of each animal.

Foot Fault

Animals were allowed to roam freely on a wire mesh grid measuring 45 × 30 cm, with square openings measuring 2.5 × 2.5 cm, and elevated 2 cm above a solid base. Trials of 50 total steps for each hind limb were recorded for each animal. A full fault was recorded if the animal's hind limb fell through the grid opening and touched the base. A partial fault was recorded if the animal's hind limb fell through the opening but did not touch the base. An FF asymmetry score was calculated using the following equation: composite FF score = (n partial faults × 1) + (n full faults × 2), %FF = (composite FF score/total number of steps) × 100%, FF asymmetry score = %FF (normal hind limb) – %FF (surgical hind limb).

Sciatic Functional Index

The hind limbs were inked, such that red designated the unoperated limb and black designated the surgical limb. Animals were placed on the beam farthest from the cage, and 6 consecutive (3 from each limb) foot prints were recorded. As previously published,^{9–11} the SFI was scored using the following parameters: normal print length (NPL), normal toe spread (NTS), normal intermediary toe spread (NIT), experimental print length (EPL), experimental toe spread (ETS), and experimental intermediary toe spread (EIT). Sciatic functional index scores were calculated using the following formula: $SFI = -38.3([EPL - NPL]/NPL) + 109.5([ETS - NTS]/NTS) + 13.3([EIT - NIT]/NIT) - 8.8$.¹²

Sciatic Nerve and Gastrocnemius/Soleus Muscle Harvest

Once the animals reached the 6-week end point, they were euthanized with Euthasol (Virbac, Fort Worth, Tex) intracardiac injections. The prior incision was reopened, and the full length of the nerve was harvested at the most proximal and distal points for histological or DTI analysis. The gastrocnemius/soleus muscles from the bilateral hind limbs were then carefully dissected off surrounding tissues and weighed (gm) to obtain a net wet weight (normal hind limb muscle weight – surgical hind limb muscle weight).

Histology

Immunohistochemistry—Immunohistochemical staining was performed using commercial antibodies specifically directed against choline acetyltransferase (Millipore, Temecula, Calif) for motor axons at 1:200 for 1 hour as previously described.¹³ Digital images of slides were acquired with an Olympus C-35AD-4 microscope at 10× using Image-Pro Plus 7.0 (Media Cybernetics, Bethesda, Md). Stained axons were counted using ImageJ (Wayne Rasband, National Institute of Mental Health, Bethesda, Md) software.

Toluidine Blue Stain—Harvested nerves were fixed in 2% paraformaldehyde/3% glutaraldehyde, postfixed with 1% OsO₄ solution, dehydrated in increasing concentrations of ethanol, and then embedded in resin at 60° for 48 hours. Samples were cut into 1-μm-thick sections and stained with 1% toluidine blue. Digital images of slides were acquired with an Olympus C-35AD-4 microscope. Axon count, density (number/mm²), and diameter (μm) were measured using Image-Pro Plus 7.0. Within each segment of each nerve, axon density was calculated by manually counting axons within 3 randomly selected areas at 40× and then averaged to avoid selection bias. Total axon count was calculated by multiplying the average axon density by the nerve cross-sectional area. Axon diameter was calculated using the average diameter of 60 randomly chosen axons.

Diffusion Tensor Imaging

Preparation—Harvested nerves were incubated in 4% glutaraldehyde/0.5% paraformaldehyde in phosphate-buffered saline at 4°C. After 24 hours postfixation, excised nerves were replaced in phosphate-buffered saline + 2mM Gd-DTPA (Magnevist, Bayer HealthCare, Wayne, NJ) at 4°C for at least 24 hours before imaging. For imaging, excised nerves were placed in 2-mm outer diameter glass capillary tubes filled with a perfluoropolyether liquid (Fomblin, Solvay Solexis, Thorofare, NJ) for susceptibility matching, preventing tissue dehydration, and a signal-free background. For higher throughput, 6 excised sciatic nerves in a hexagonal arrangement were imaged simultaneously.

Diffusion Tensor Imaging Acquisition—Magnetic resonance imaging was performed on a 4.7-T 31-cm horizontal bore Agilent DirectDrive scanner (Agilent Technologies, Santa Clara, Calif) using a 38-mm Litz quadrature coil (Doty Scientific, Columbia, SC) for radiofrequency transmission and reception. Diffusion tensor imaging data were acquired using a 3-dimensional (3D) diffusion-weighted spin echo sequence with repetition time/echo time = 170/ 23.0 ms, 12 signal averages, field-of-view = 9.6 × 9.6 × 14.4 mm³, and matrix size = 96 × 96 × 32 for a nominal resolution of 100 × 100 × 450 μm³ (450 μm along the

nerve). Diffusion weighting was achieved with $\delta/\gamma = 4/12$ ms, a prescribed b value of 2000 s/mm^2 , and 6 directions. One $b = 0$ image was acquired for a total of 7 images in a scan time of approximately 12 hours.

Diffusion tensor imaging Analysis—Image data reconstruction was performed using in-house written code in MATLAB (MathWorks, Natick, Mass), and DTI analysis was implemented in ExploreDTI.¹⁴ 3D image volumes were zero padded 2 times in each direction during reconstruction from k-space data. Diffusion tensors were estimated voxel-wise using a linear least-squares approach. From the diffusion tensor, DTI metrics including FA, AD, and RD were computed voxel-wise and DTI tractography was performed. Then, DTI parameters were measured for 3 regions of interest (ROIs): 1.5 mm proximal to the proximal stump, the middle of the autograft, and 1.5 mm distal to the distal stump.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics 23.0 software (IBM Corp, Armonk, NY). Statistical significance was determined using nonparametric statistics, Mann-Whitney U , or Kruskal-Wallis H test. Values are reported as median [interquartile range (IQR)]. Statistical significance was assigned a P value of less than 0.05.

RESULTS

Behavioral Evaluations of Sciatic Nerve Function

At all test time points, reverse and normally oriented autografts demonstrated similar values in both FF and SFI scores. The sham group, however, consistently demonstrated noninjured behavioral patterns compared with both autograft groups (Fig. 1A, B).

Gastrocnemius/Soleus Net Wet Weights

Muscle net wet weights in reverse and normally oriented autografts did not differ [1.36 g (IQR, 1.30–1.44) vs 1.33 g (IQR, 1.24–1.37); $P = 0.68$], although were significantly more atrophied than shams (0.02 g; IQR, –0.7 to 0.03; $P < 0.05$).

Nerve Histology With Immunohistochemistry

After staining with choline acetyltransferase for motor axons, cross sections proximal to, within, and distal to the graft were evaluated. Animals demonstrated no difference in motor axon counts at each segment between reverse and normally oriented autografts. Figures 2 and 3 demonstrate motor axon counts and representative slides within each group at any given nerve segment, respectively.

Nerve Histology With Toluidine Blue Axon Staining

We observed morphologically normal axons with interspersed regenerative patterns and edema, 1.5 mm proximal to the proximal injury site in both autograft groups. The total number of axons within the grafts in the reverse autograft group was 8429 (IQR, 8274–8762), and 8630 (IQR, 6353–8982) axons in the normally oriented autograft group ($P = 0.57$). Axon density was 7429 axons/ mm^2 (IQR, 7291–7722) in the reverse group, with a density of 7739 axons/ mm^2 (IQR, 7470–7951) in normally oriented autografts ($P = 0.57$).

Axon diameter was 5.09 μm (IQR, 4.64–5.52) versus 5.16 μm (IQR, 4.97–5.83), respectively ($P = 1.00$). Distally along the length of the nerve in both autograft groups, axon density decreased as did axon diameter. In the distal segment, the axon count was 7668 (IQR, 7010–7773) and 7547 (IQR, 6715–7836) ($P = 1.00$) in the reverse and normal autografts, respectively. Axon density was 5905 axons/ mm^2 (IQR, 5398–5985) in reverse autografts, with an axon density of 5812 axons/ mm^2 (IQR, 5171–6034) in the normally oriented autografts ($P = 1.00$). The diameter of axons were 3.92 μm (IQR, 3.67–4.16) in the reverse autograft group and 3.51 μm (IQR, 3.20–4.19) in the normally oriented group ($P = 1.00$). Shams had an axon count of 6778 (IQR, 6197–7212), density of 6655 axons/ mm^2 (IQR, 6084–7081), and diameter of 8.74 μm (IQR, 8.45–9.17). Representative images are seen in Figure 4.

Diffusion Tensor Imaging and Tractography

Fractional anisotropy, AD, and RD were measured for each ROI. Diffusion tensor imaging measures comparing reverse and normally oriented autografts were comparable, signifying similar axonal regeneration. As such, there was no statistically significant difference in FA, AD, and RD at any segment when comparing reverse and normally oriented autograft to each other. When comparing shams to autografts, both reverse and normally oriented autografts demonstrated a statistically significant decrease in FA at ROIs within and distal to the graft compared with shams. This decrease in FA was also evident at the proximal segment of the autografts in comparison to shams, which corresponded to edematous findings on toluidine blue histological analysis. Axial diffusivity was similar, although RD was significantly increased in the autograft groups compared with shams. See Table 1 for a complete comparison of DTI values between sham and autograft groups. Diffusion tensor imaging tractography displayed proximodistal regenerative tracts in both reverse and normally oriented autograft groups (Fig. 5).

DISCUSSION

Few experiments have been performed to explore the effect of autograft orientation during peripheral nerve repair, with the most recent being more than a decade ago. Each study has been typically limited to very few assessment tools to compare reverse and normally oriented autografts. Furthermore, results between different studies have been inconsistent, therefore leading to ambiguity in the proper autograft orientation that would result in the best regenerative outcome. Stromberg et al¹ performed the first study in a rat model to assess nerve autograft polarity in 1979. Their study demonstrated outcomes were independent of polarity of the nerve graft. Nerve conduction velocity and amplitude, as well as histological analyses, were indistinguishable between reverse and normally oriented autografts at 6 months. Similar findings were demonstrated in a rabbit model, using 2-cm cable grafts.³ In our study, histological analysis at 6 weeks, whether by immunohistochemistry or toluidine blue staining, likewise demonstrated similarity in quantitative axonal measures such as axon count, density, and diameter in reverse and normally oriented autografts. Therefore, both short- and long-term axonal regeneration appear to be similar irrespective of nerve autograft polarity. Concordant with equivalent axonal growth, in our study a comparable degree of muscle atrophy was observed in the affected limb between the autograft groups, which was

also evident in a 6-month study by Nakatsuka et al³ in a rabbit model. Ansselin and Davey, in their rat experiment using fibularis or tibialis nerves, found that nerve regeneration within the autograft was similar using either orientation; however, the distal cross-sectional area in the normally oriented autografts was reduced. They observed that axonal regeneration occurs through terminal nerve branch points in the normally oriented autografts, whereas they “disappear” in reverse autografts. From these findings, they concluded that some axonal regeneration is lost distally through terminal branches in normal grafts.^{4,5} The studies by Ansselin and Davey only provided histological analysis, without offering other assessments such as animal behavior, but nonetheless have been the cornerstone of why some advocate reversing the orientation when using autografts with branch points.

Sotereanos et al² studied nerve polarity in a nonbranched rat sciatic nerve model using the Bain modification of de Medinaceli's SFI behavioral study to assess functional outcomes. Functional recovery and histological analysis of regeneration were found to be equivalent regardless of autograft orientation. Dellon and Mackinnon¹⁵ have shown SFI to be a very useful tool after nerve injury. The similar weekly SFIs demonstrated up to 6 weeks in our study, and up to 120 days in Sotereanos et al study evaluating SFI between reverse and normally oriented autografts, suggest similar short- and long-term functional outcomes.² Foot fault, a behavioral injury assessment model described for sensorimotor recovery, particularly for up to 30 to 60 days postinjury,¹⁶ also showed similar recovery patterns up to 6 weeks.

The uncertainty regarding the best autograft orientation is evident due to discrepancies in findings between studies by Ansselin and Davey in branched models with other published literature. Furthermore, despite some evidence of similar outcomes between reverse and normally oriented autograft in nonbranched models, proper autograft orientation remains ambiguous. Some advocate the use of reverse autografts due to the concerns of losing axons through major branches,^{4,5,17} whereas some advocate keeping the normal physiologic orientation to either to maintain physiologic axoplasmic flow¹⁸ or because reverse autografts do not overcome the branch point problem.¹⁹ Others either identify that there is no difference¹⁻³ or do not make mention of a suggested orientation.

In this study, we used DTI to assess axonal regeneration. Previous studies have evaluated peripheral nerves with DTI metrics and compared them to histological findings and animal behavior scoring.²⁰⁻²² Lehmann et al⁶ found that there was a positive correlation between FA value and the total number of myelinated axons after crush injury in the mouse model. Various other studies have had similar results, validating DTI as an appropriate tool in imaging peripheral nerve injury models.^{6,20-22} This advanced magnetic resonance imaging technology characterizes and quantifies diffusion of water in a tissue of interest (ie, nerves) and reflect alterations in tissue microstructure. Isotropic diffusion refers to diffusion that is equal in all directions, as would be seen in a 3D sphere. However, nerve tissue is constrained by myelinated fibers and fascicles, causing diffusion of water along a dominant pathway, termed anisotropic diffusion. Typically, AD and RD are used to derive quantitative DTI parameters. Axial diffusivity refers to the extent of water molecules parallel to the direction of nerve fibers, whereas RD is the extent of diffusion of water molecules perpendicular to the direction of nerve fibers. The values are used to compute FA, an index of how different

the principal diffusions are, with values ranging from 0 to 1. A value closer to 0 corresponds to random, multidirectional diffusion of water, whereas a value closer to 1 indicates that the nerve is directing diffusion strictly along one direction in its axis (anisotropy)⁶ The FA for a normal, uninjured nerve is expected to be between 0.6 and 0.8, where axons are aligned relatively linearly, as FA fails to reach the value of 1 due to the presence of structures such as glial cells, axons, Schwann cells, myelin, etc, which can provide a barrier to complete anisotropic diffusion.^{8,23} Nerve injury is associated with less directionality, as, for example, myelin disruption causes unorganized diffusion, thus exhibiting lower values of FA. In this study, FA values for shams were similar to those of other uninjured models in previous studies and were higher than those in the autograft groups. Although some studies have demonstrated that RD is a useful nerve monitoring parameter, most studies agree that changes in FA correlate with peripheral nerve regeneration.^{8,20,22,24} Morisaki et al's²² study of crush injury in a rat sciatic nerve model demonstrated decreased FA and increased RD in the degenerative phase up to 3 weeks after injury. This is similar to Yamasaki et al's²⁰ study on crush injury of rabbit sciatic nerve where a decreased FA was observed until 4 weeks postinjury, representing lowered anisotropic diffusion due to the loss of nerve integrity. These time points represent the end of Wallerian degeneration and the continued clearance of myelin and axonal debris. At 6 weeks, we observed a higher FA in the autograft groups compared with the findings of Morisaki et al and Yamasaki et al at earlier time points. This indicates that at 6 weeks, the autograft groups have entered the axonal regeneration phase, however incompletely, as FA levels have not reached values of shams. Radial diffusivity was also significantly increased in autograft groups compared with shams, as demonstrated in previous DTI studies, though inconsistently. The comparison of axonal regeneration, however, proved to be similar between the autograft groups, as the degrees of FA, RD, and AD were similar regardless of orientation. Diffusion tensor imaging data in this study comparing autograft to shams, and autografts with each other are consistent with the behavioral and histological results observed in our experiment. Furthermore, our DTI results are comparable to outcomes in previous DTI studies in crush-injured and non-gap nerve injury repair models. These findings suggest that DTI may be a useful tool in evaluating and assessing nerve injury, including in nerve gap models. It is important to note that all studies, including the current study, comparing reverse and normally oriented autografts are in animal models due to the inability to provide histological analysis in human models. Perhaps, DTI may be a useful noninvasive tool to provide objective data in human nerve models.

The results of this study suggest that autograft orientation may have no bearing on functional or histological outcomes for at least 6 weeks postoperatively. This time point was chosen to minimize the effect of enhanced neuroregenerative capacity seen in rat models, as experiments with later time points may artificially result in similar outcomes regardless of the type of treatment.²⁵ Furthermore, as DTI measures begin improving after 3 to 4 weeks in crush injury models, our goal was to capture any early difference, as these measures have never been explored in autograft models. Longer term DTI outcomes need to be further explored in future studies.

Although it has been previously described that some regenerating axons may be lost in terminal branches, branches have also been shown to “disappear” or collapse^{4,5}; therefore,

branching in the graft may have minimal effect. Our study does not evaluate the effect of branch points and, therefore, cannot attest to its consequence. Under conditions where nonbranching autografts are used, orientation was not shown to influence functional or regenerative measures. Although branched autografts (ie, sural nerve) are more commonly used than nonbranched, there has been a gain in popularity of allograft use for nerve repairs. Their orientation is usually unknown, and therefore, the results of this study may provide reassurance that outcomes are similar regardless of orientation. Future studies should investigate the outcomes of reverse and normal autografts with branch points using a comprehensive list of assessment tools including DTI, and perhaps in clinically comparable larger sized nerve models, including human models, to investigate whether disappearance or collapse of branches occurs and how this may or may not affect outcomes.

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FIGURE 1.

A, Sciatic function index scores for shams, normal, and reverse orientated autografts at 72 hours and weekly postoperative time points. A more negative score indicates more impairment. B, FF asymmetry scores for shams, normal, and reverse orientated autografts at 72 hours and weekly postoperative time points. A more negative score indicates more impairment.

FIGURE 2.

Motor axon counts using immunohistochemistry at low magnification ($\times 10$) for shams, normal, and reverse orientated autografts. Motor axon counts for normal and reverse oriented autografts were evaluated at segments proximal, within, and distal to the graft site, whereas only at 1 segment for shams.

FIGURE 3.

Representative images of immunohistochemical choline acetyltransferase stain for motor axons at low magnification ($\times 10$) for shams, normal, and reverse orientated autografts. Motor axons for normal and reverse oriented autografts were evaluated at segments proximal, within, and distal to the graft site, whereas only at 1 segment for shams as a reference. No difference was observed in motor axon count between normal and reverse oriented autografts.

FIGURE 4.

Representative images of toluidine blue axon stains of 1- μ m-thick section at $\times 40$ magnification for shams, normal, and reverse orientated autografts. Axons for normal and reverse oriented autografts were evaluated at segments proximal, within, and distal to the graft site, whereas only at 1 segment for shams as a reference. No difference was observed in total axon count, axon density, or diameter between normal and reverse oriented autografts.

FIGURE 5.

Diffusion tensor imaging tractography images at 6 weeks demonstrating nerve track regeneration for (A) sham, (B) normal orientated, and (C) reverse orientated autografts. Normal and reverse autografts both demonstrate proximodistal regeneration, however with less linear directionality compared with shams.

TABLE 1

Diffusion Tensor Imaging Parameters in Each Segment Between Normally Oriented Autografts (n = 6), Reverse Oriented (n = 6) Autografts, and Shams (n = 6) at 6 Weeks, Demonstrating Statistically Significant Difference in FA and RD at All Segments Between Shams and Autografts, But No Difference in AD Values

	Proximal	IQR	P	Graft	IQR	P	Distal	IQR	P
FA									
Sham	0.70	0.68–0.72							
Normal orientation	0.55	0.49–0.57	<0.05	0.59	0.50–0.64	<0.05	0.56	0.48–0.61	<0.05
Reverse orientation	0.53	0.49–0.56		0.55	0.53–0.60		0.54	0.51–0.58	
AD, $\mu\text{m}^2/\text{ms}$									
Sham	0.82	0.80–0.85							
Normal orientation	0.82	0.78–0.83	0.75	0.78	0.69–0.79	0.06	0.77	0.75–0.78	0.08
Reverse orientation	0.81	0.76–0.87		0.76	0.75–0.83		0.77	0.76–0.82	
RD, $\mu\text{m}^2/\text{ms}$									
Sham	0.24	0.22–0.25							
Normal orientation	0.34	0.34–0.37	<0.05	0.32	0.29–0.34	<0.05	0.34	0.32–0.37	<0.05
Reverse orientation	0.38	0.34–0.44		0.34	0.31–0.39		0.35	0.33–0.40	

Statistical significance, $P < 0.05$.

P value comparing sham, normal orientation, and reverse orientation groups using Kruskal-Wallis H .

Note: No statistical difference in FA, AD, or RD at any segment when normal orientation and reverse orientation autografts are compared with each other (not shown).