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The impact of motor and sensory nerve architecture on nerve regeneration

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ABSTRACT

Sensory nerve autografting is the standard of care for injuries resulting in a nerve gap. Recent work demonstrates superior regeneration with motor nerve grafts. Improved regeneration with motor grafting may be a result of the nerve's Schwann cell basal lamina tube size. Motor nerves have larger SC basal lamina tubes, which may allow more nerve fibers to cross a nerve graft repair. Architecture may partially explain the suboptimal clinical results seen with sensory nerve grafting techniques. To define the role of nerve architecture, we evaluated regeneration through acellular motor and sensory nerve grafts. Thirty-six Lewis rats underwent tibial nerve repairs with 5 mm double-cable motor or triple-cable sensory nerve isografts. Grafts were harvested and acellularized in University of Wisconsin solution. Control animals received fresh motor or sensory cable isografts. Nerves were harvested after 4 weeks and histomorphometry was performed. In 6 animals per group from the fresh motor and sensory cable graft groups, weekly walking tracks and wet muscle mass ratios were performed at 7 weeks. Histomorphometry revealed more robust nerve regeneration in both acellular and cellular motor grafts. Sensory groups showed poor regeneration with significantly decreased percent nerve, fiber count, and density ($p<0.05$). Walking tracks revealed a trend toward improved functional recovery in the motor group. Gastrocnemius wet muscle mass ratios show a significantly greater muscle mass recovery in the motor group ($p<0.05$). Nerve architecture (size of SC basal lamina tubes) plays an important role in nerve regeneration in a mixed nerve gap model.

Introduction

Sensory nerve autografting is the accepted technique for reconstruction of peripheral nerve defects despite suboptimal outcomes (Kline, 1990; Mackinnon and Dellon, 1988; Mackinnon et al., 2001; Sunderland, 1978; Terzis and Smith, 1990). Recent studies suggest that modality specific regeneration (MSR) may influence nerve regrowth. Brushart et al. demonstrated that regenerating motor axons preferentially choose the motor pathway (Preferential Motor Reinnervation, or PMR) following injury (Brushart, 1988; Madison et al., 1996). Motor nerve grafts result in a four fold increase in nerve total fiber counts compared to sensory nerve grafts (Brenner et al., 2006; Nichols et al., 2004). Possible governing mechanisms for this phenomenon include differences in biochemical markers, Schwann cell phenotype, neurotrophic support, and/or nerve architecture (Brenner et al., 2006; Brushart, 1988, 1993; Brushart and Seiler, 1987; Ghalib et al., 2001; Martini et al., 1994, 1992; Nichols et al., 2004).

Differences in neurotrophic factor presence and expression have been identified in motor and sensory nerves: insulin-like growth factor (Caroni and Grandes, 1990; Hansson et al., 1986), basic fibroblast growth factor (Danielsen et al., 1988; Grothe et al., 1991), and ciliary neurotrophic factor (Arakawa et al., 1990; Forger et al., 1993; Manthorpe et al., 1986; Oppenheim et al., 1991), are all believed to affect motor nerve phenotypes. Other studies evaluating the sciatic and facial nerves demonstrated that the presence or application of glial cell line-derived neurotrophic factor and neurturin reduced the amount of axonal cell death, and promoted motor nerve regeneration (Fine et al., 2002; Florian et al., 2002; Hoke et al., 2002; Bohn, 2004; Oppenheim et al., 2000).

Repair of a tibial nerve defect with motor, sensory, and mixed nerve grafts demonstrated significantly improved regeneration through motor nerve cable grafts (femoral motor nerve to the quadriceps) compared to sensory nerve cable grafts (femoral cutaneous nerve) (Nichols et al., 2004). This held true even if a single cable of motor nerve was used (Brenner et al., 2006). However, when nerve graft architecture was disrupted and nerve cellularity was maintained (i.e., Schwann cells), the benefit seen with motor graft material was lost (Lloyd et al., 2007). Neurotrophic differences alone do not completely account for the differences in regeneration observed with motor and sensory nerve grafting.

Previous studies have demonstrated that regenerating axons prefer to grow along Schwann cell basal lamina tubes (Ramon y Cajal, 1928), and intact nerve architecture allows the basal lamina tubes to guide

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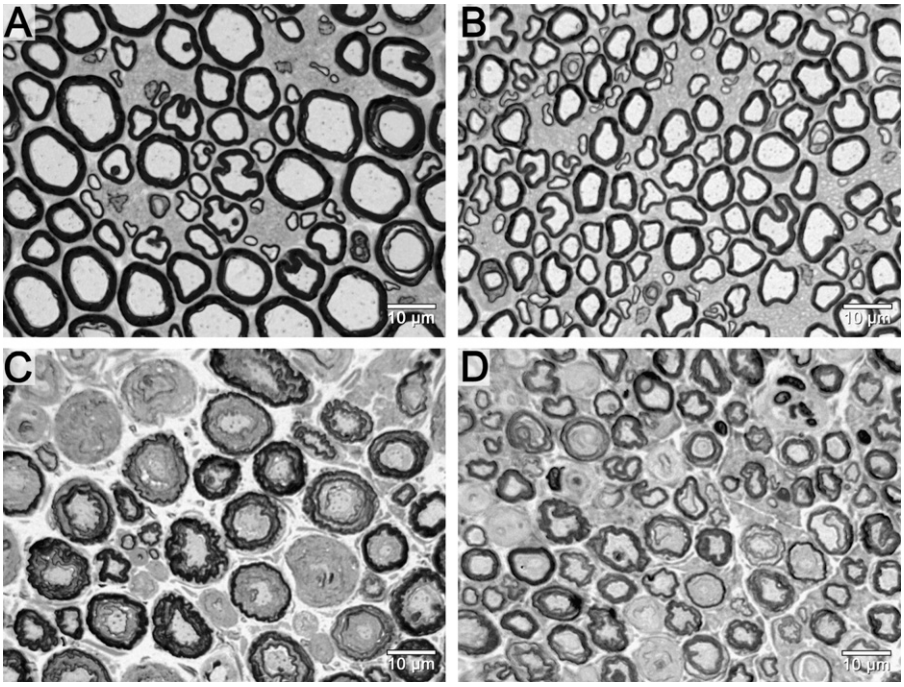

Fig. 1. Photomicrographs of femoral motor and sensory nerve cross-sections. The fresh motor nerve (A) has larger diameter, but, fewer fibers in comparison to the fresh sensory nerve (B). These larger endoneurial tubes may mechanically provide a more favorable size conduit for nerve regeneration than the smaller sensory endoneurial tube. Comparisons between fresh motor (A) and cold motor (C) or fresh sensory (B) and cold sensory (D) nerves demonstrate the small amount of shrinkage that takes place during the cold preservation procedure. Scale bars in the bottom right hand corner.

regenerating axons to the desired distal target (Brown and Hopkins, 1981). The Schwann cell basal lamina tubes than contain motor fibers are larger than those that contain sensory fibers (Figs. 1A and B). The present study tests the hypothesis that architectural differences between motor and sensory nerves confer the regeneration advantage seen with motor nerve grafting in a mixed nerve gap model.

Materials and methods

Nerves were decellularized in University of Wisconsin organ preservation solution for 7 weeks, leaving intact, the basal lamina tube architecture and laminin in the extracellular membrane (Evans et al., 1998). After 7 weeks of preservation, nerves lack immunogenicity and can support regeneration in a short nerve gap model (Fox et al., 2005).

Experimental design

We assigned 12 animals randomly to each of 2 graft recipient groups: fresh motor and fresh sensory and 6 animals to each of 2 graft recipient groups: cold motor and cold sensory (Table 1). An additional 18 animals were used as nerve donors.

Table 1
Experimental design

Group	N	Description	Outcome measure(s)	Endpoint
I	12	Fresh Motor Isograft (double-cable)	6: Histomorphometry 6: Weekly walking tracks, wet muscle mass ratios	4 weeks 7 weeks
II	12	Fresh Sensory Isograft (triple-cable)	6: Histomorphometry 6: Weekly walking tracks, wet muscle mass ratios	4 weeks 7 weeks
III	6	Cold Preserved Motor Isograft (double-cable)	All: Histomorphometry	4 weeks
IV	6	Cold Preserved Sensory Isograft (triple-cable)	All: Histomorphometry	4 weeks

Animals in group I received 5 mm motor (femoral motor branch to quadriceps) isografts, animals in group II received 5 mm sensory (femoral cutaneous branch) isografts, animals in group III received 5 mm cold preserved (decellularized) motor isografts, and animals in group IV received 5 mm cold preserved sensory isografts. The choice of 5 mm motor and sensory grafts stems from the availability of donor nerve. Specifically, the rat femoral motor nerve can reliably provide 5 mm of length without significant neurolysis. A longer graft such as 1 cm grafts used in other studies requires significant neurolysis and may include sensory fibers in the motor donor graft.

Due to the differences in nerve diameters and cross-sectional area (Brushart and Seiler, 1987), the size disparity between sensory and motor nerves was normalized by constructing a triple cable sensory graft and a double cable motor graft. This arrangement provides grafts of comparable diameter and area (Nichols et al., 2004).

In the rodent model, timing of evaluation is critical because of the superlative regenerative capacity of these animals. Early evaluation prevents “blow-through”, where true differences in histological recovery are masked by robust regeneration not seen in humans (Brenner et al., 2008). Because of these considerations, a four-week endpoint was used to evaluate histological recovery. A seven-week endpoint was then chosen to provide information on functional recovery, which lags histological findings since muscle reinnervation and function is required.

Methods

Adult male Lewis rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 310 to 410 g were housed in a central animal facility, given a rodent diet (PicoLab Rodent Diet 20 #5053, PMI Nutrition International) and water ad libitum. All surgical procedures, experimental manipulations, and peri-operative care measures were carried out in strict accordance with National Institutes of Health guidelines and were approved by the university institutional Animal Studies Committee. Animals were returned to the animal facility following surgical procedures and monitored for infection, weight loss, or other disability.