INTRODUCTION: Schwann cells are routinely described as expressing one of two possible phenotypes, myelinating or non-myelinating. We now present evidence that Schwann cells also express distinct sensory and motor phenotypes, and that these phenotypes are associated with modality-specific promotion of axon regeneration.

A functional difference between sensory and motor nerve was unmasked by experiments designed to provide regenerating motor axons with equal access to both environments. Under these conditions, motor axons preferentially reinnervated muscle pathways, even when these pathways had been blocked off distally to prevent axons from reaching muscle (Brushart, 1993). These motorneurons were also found to support significantly more myelinated collaterals in cutaneous nerve than in muscle nerve, indicating a modality-specific difference in the ability of pathways to stimulate and/or maintain collateral sprouts (Redett et al., 2005). Cutaneous and muscle nerve thus differ in ways that can be detected by regenerating motor axons and that can significantly modify their behavior.

METHODS: Four experimental configurations were generated to reinnervate cutaneous nerve graft with cutaneous axons (Cutaneous axons, Cutaneous graft; C-C) or motor axons (M-C), and to similarly reinnervate grafts of ventral root with cutaneous axons (C-V) or motor axons (M-V). Experiments were performed in Lewis rats after approval of the Johns Hopkins Animal Care and Use Committee. A pure source of sensory axons was obtained by transecting the femoral cutaneous nerve. A pure population of motor axons was obtained by unilateral excision of the L3, L4, L5, and L6 dorsal root ganglia followed by sciatic nerve transection. To obtain grafts, the femoral cutaneous nerve was transected proximally at the femoral bifurcation, where it is unifascicular, and the L3, L4, L5, and L6 dorsal root ganglia followed by sciatic nerve sensory axons was obtained by transecting the femoral cutaneous nerve. A pure population of motor axons was obtained by unilateral excision of the L3, L4, L5, and L6 dorsal root ganglia followed by sciatic nerve transection. To obtain grafts, the femoral cutaneous nerve was transected proximally at the femoral bifurcation, where it is unifascicular, and again 2 cm distally. Two-centimeter lengths of L4 and L5 ventral root grafts were harvested from donor rat muscles when sacrificed.

Denervated graft controls were designed to permit normal graft revascularization and Wallerian degeneration, but without axon ingrowth. Cutaneous nerve grafts were sewn to the femoral cutaneous nerve. Experiments were performed in Lewis rats after approval of the Johns Hopkins Animal Care and Use Committee. A pure source of sensory axons was obtained by transecting the femoral cutaneous nerve. A pure population of motor axons was obtained by unilateral excision of the L3, L4, L5, and L6 dorsal root ganglia followed by sciatic nerve sensory axons was obtained by transecting the femoral cutaneous nerve. A pure population of motor axons was obtained by unilateral excision of the L3, L4, L5, and L6 dorsal root ganglia followed by sciatic nerve transection. To obtain grafts, the femoral cutaneous nerve was transected proximally at the femoral bifurcation, where it is unifascicular, and again 2 cm distally. Two-centimeter lengths of L4 and L5 ventral root grafts were harvested from donor rat muscles when sacrificed.

Competitive RT-PCR was performed on normal cutaneous nerve and ventral root, and on each of the 6 graft preparations 5 days, 15 days, and 30 days after surgery, yielding a total of 20 groups of nerves. Twelve grafts were set up for each group, requiring a total of 340 surgical preparations for PCR analysis. Genes of interest included NGF, BDNF, GDNF, PTN, and related light chain. Total RNA was extracted from the nerves using Trizol (Invitrogen). The cDNA was synthesized using 2 µg of total RNA in the presence of Ready-to-Go cDNA synthesis kit (Amersham) and random primers (Invitrogen). PCR amplification and the absence of nonspecific bands. The RT-PCR experiments were performed by real-time RT-PCR using two-color DNA Engine Opticon System (MJ Research Inc.) and the relative amount of gene of interest was normalized to the mRNA amount of an internal control (GAPDH) in the same PCR reaction. To avoid the possibility of amplifying contaminating DNA, all of the primers for real-time RT-PCR were designed with an intron sequence inside the cDNA to be amplified; reactions were performed with appropriate negative control samples (template-free control samples); a uniform amplification of the products (dissociation graphs); the melting temperature (Tm) was 57°C to 60°C; the probe Tm was at least 10°C higher than primer Tm; and gel electrophoresis was performed to confirm the correct size of the amplified products. Amplification of the products was rechecked by analyzing the melting curves of the amplified products.

Graft reinnervation was assessed in groups C-C, C-V, M-C, and M-V after 2 weeks of regeneration, as trophic factor expression usually peaked at that time. The graft was crushed with a narrow microforcep 1 cm from the host-graft juncture, and the fluorescent tracer Fluoro-Ruby (Molecular Probes, Eugene, Oregon) was injected into the crush zone. Forty-eight hours later the animals were perfused with 4% paraformaldehyde. Forty-micron sections of spinal cord or L2, L3, and L4 DRG’s were cut on a freezing microtome and viewed with fluorescent light (555 nm excitation, 580 nm emission) by an observer unaware of the graft type used. All nuclei within labeled neurons were counted, and the presence of split nuclei was corrected for as described by Abercrombie (1946). Counts were compared by unpaired t-test analysis.

RESULTS: PCR analysis of groups NO-C and NO-V revealed that NGF, BDNF, IGF-1, HGF, and VEGF were significantly upregulated in denervated cutaneous nerve, but minimally if at all in denervated ventral root. GDNF and PTN, in contrast, were upregulated by both denervated ventral root and cutaneous nerve. Minimal upregulation in ventral root after denervation accurately predicted a similar response after reinnervation by either cutaneous or motor axons. Schwann cells of ventral root are thus not induced to produce these factors during the period of study, no matter what their axonal partners. These same factors also responded variably to reinnervation of cutaneous nerve. For instance, upregulation of NGF in denervated cutaneous nerve peaked at 15 days and returned towards baseline by 30 days. When the nerve was reinnervated by either motor or sensory axons, however, downregulation of NGF occurred by day 15, and thus potentially in response to axonal contact. GDNF and PTN were upregulated by both cutaneous nerve and ventral root, but more vigorously by the latter. In cutaneous nerve, GDNF and PTN responded only to cutaneous axons, with transient upregulation of GDNF at day 5 and a low, but more sustained upregulation of PTN. Reinnervation of ventral root by cutaneous axons caused prolonged expression of GDNF but only a brief burst of PTN expression, while reinnervation by motor axons had little effect on GDNF but occasioned a more sustained expression of PTN.

Regeneration in the four experimental groups was assessed by retrograde labeling to ensure that significant axon ingrowth had occurred, and to compare the performance of the four combinations of axon and pathway. After 2 weeks of regeneration, a mean of 1188 (SE 120) DRG neurons projected axons 1 cm down the graft when it was cutaneous nerve (C-C), but a mean of only 423 (SE 61) extended this far when it was ventral root (C-V) (p<0.0001). Conversely, a mean of 398 (SE 28) motoneurons projected their axons 1 cm down the graft when it was ventral root (M-V), but a mean of only 151 (SE 16) reached this far when it was cutaneous nerve (M-C) (p<0.0001). Cutaneous nerve thus selectively promotes the regeneration of sensory axons, while ventral root selectively promotes the regeneration of motor axons.

DISCUSSION: The RT-PCR data obtained in these experiments substantiate our hypothesis that Schwann cells of cutaneous nerve and ventral root respond differently to denervation and reinnervation. Five factors - NGF, BDNF, VEGF, HGF, IGF-1 - were upregulated vigorously by cutaneous nerves, whereas only PTN and GDNF were upregulated by ventral root. Within this group, the response of cutaneous nerve to denervation and reinnervation by appropriate or inappropriate axons also varied significantly. Two factors, GDNF and PTN, were upregulated substantially by both cutaneous nerve and ventral root. However, the 10-fold higher baseline expression of GDNF in cutaneous nerve tends to equalize the final expression of GDNF in the two environments. The 100-fold higher baseline expression of PTN in fresh ventral root, coupled with substantial further upregulation, suggests that PTN is predominately a motor factor. Matching pure populations of cutaneous and motor axons with homogenous groups of cutaneous or motor Schwann cells provides a clear test of axon-Schwann cell interactions. Under the current experimental conditions, cutaneous Schwann cells preferentially support cutaneous axon regeneration, and ventral roots preferentially support motor axon regeneration. For the last century, cutaneous nerve has been used almost exclusively for clinical nerve reconstruction. The present findings thus help to explain why the results of grafting motor nerves consistently lag behind those of end-to-end nerve repair, and suggest strategies to improve outcome by manipulating Schwann cell phenotype.