Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 53–57, January 1994
Neurobiology

Transplantation of glial cells enhances action potential conduction of amyelinated spinal cord axons in the myelin-deficient rat

DAVID A. UTZSCHNEIDER*, DAVID R. ARCHER†, JEFFERY D. KOCSIS*‡, STEPHEN G. WAXMAN*, AND IAN D. DUNCAN†

*Department of Neurology and the Neuroscience Program, Yale University Medical School and Neuroscience Research Center, VA Medical Center, West Haven, CT 06516; and †Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706-1102

Communicated by Francis O. Schmitt, September 13, 1993

ABSTRACT **A central issue in transplantation research is to determine how and when transplantation of neural tissue can influence the development and function of the mammalian central nervous system. Of particular interest is whether electrophysiological function in the traumatized or diseased mammalian central nervous system can be improved by the replacement of cellular elements that are missing or damaged. Although it is known that transplantation of neural tissue can lead to functional improvement in models of neurological disease characterized by neuronal loss, less is known about results of transplantation in disorders of myelin. We report here that transplantation of glial cells into the dorsal columns of neonatal myelin-deficient rat spinal cords leads to myelination and a 3-fold increase in conduction velocity. We also show that impulses can propagate into and out of the transplant region and that axons myelinated by transplanted cells do not have impaired frequency–response properties. These results demonstrate that myelination following central nervous system glial cell transplantation enhances action potential conduction in myelin-deficient axons, with conduction velocity approaching normal values.**

The ability of transplanted glial cells to ensheath axons and synthesize myelin when transplanted into dysmyelinating mutants or chemically created models of demyelination has been studied extensively (1–6). Until now the primary assay of myelin-forming grafts has of necessity been anatomical rather than functional or electrophysiological. However, the formation of myelin by transplanted cells does not in itself ensure secure impulse conduction. Impulse conduction after glial cell transplantation depends not only on myelination but on deployment of adequate numbers and types of ion channels at the newly formed nodes of Ranvier (7–9). Moreover, incomplete or patchy remyelination might be expected to lead to conduction failure due to impedance mismatch, which occurs at junctions between myelinated and nonmyelinated axon regions (10, 11); similarly, failure to form mature paranodal axoglial junctions can shunt action current and interfere with conduction (12, 13). In the present experiments, we transplanted central nervous system (CNS) myelin-forming cells into the spinal cord of the myelin-deficient (md) rat, a mutant that lacks CNS myelin, and studied conduction properties of the axons in the region of transplantation. The absence of host myelin in this system permits positive confirmation that functional changes seen after transplantation are due to the transplanted cells and not to background host myelination (2, 3). Electrophysiological studies from the md rat spinal cord have shown that while the amyelinated fibers of the md rat are capable of secure impulse conduction, they do so at ≈1/4 the conduction velocity of age-matched controls (14). We report here that myelination of md spinal cord axons following transplantation of cultured glial cells results in a significant increase in conduction velocity, which approaches normal values.

METHODS

Female littermates of the animals to be transplanted were killed and their spinal cords were removed in a sterile manner. The spinal cords were dissected free of the meninges, chopped into small pieces, and incubated in 0.25% trypsin/0.05% DNase for a maximum of 90 min. The tissue was triturated through a flame-narrowed glass pipette until a single cell suspension was obtained. This suspension was added to a Percoll/sucrose solution (final concentrations, 25% and 0.08 M, respectively) and centrifuged at 30,000 × *g* for 45 min. The appropriate cell layer was removed and placed in culture overnight before being collected and concentrated at 50,000 cells per μl. Each recipient rat was anesthetized with halothane and a dorsal laminectomy was performed at the thoracolumbar junction. One microliter of cells was injected via a glass micropipette into two or three sites along the dorsal columns of the spinal cord. The transplant sites were marked with sterile charcoal before closing the incision. At the end of the recording session, the spinal cords were immersed in 4% glutaraldehyde in 0.1 M Sorensen's buffer, processed as described (4), and embedded in Epon. One-micrometer sections were cut and stained with toluidine blue for viewing at the light microscope level.

The transplanted rats were sacrificed for physiological experiments on postnatal day 20, 21, or 22, which is 15–17 days after the transplantation procedure. After deep pentobarbital anesthesia (60 mg/kg) the animal was perfused through the heart with ≈40 ml of a chilled, oxygenated (95% O_2/5% CO_2), high sucrose solution (containing 124 mM sucrose, 26 mM $NaHCO_3$, 3 mM KCl, 1.3 mM NaH_2PO_4, 2 mM $MgCl_2$, 10 mM dextrose) after which the spinal cord was rapidly removed en bloc and allowed to incubate and come to room temperature in the same oxygenated high sucrose solution over 30–45 min. The spinal cord was then positioned in a standard brain slice chamber perfused with artificial cerebrospinal fluid (containing 124 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 1.3 mM NaH_2PO_4, 2 mM $MgCl_2$, 10 mM dextrose, 2 mM $CaCl_2$) and incubated for a further 30 min before recording field potentials. After gentle removal of the dorsal meninges overlying the transplantation site, stimulation of the dorsal columns was obtained with bipolar electrodes placed lightly on the dorsal surface of the spinal cord. Field potentials were recorded with glass microelectrodes (1–5 MΩ; 1 M NaCl) attached to a high input impedance amplifier and stored on a digital oscilloscope. Field potentials

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CNS, central nervous system; md, myelin deficient.

‡To whom reprint requests should be addressed at: Neuroscience Research Center (127A), VA Medical Center, West Haven, CT 06516.

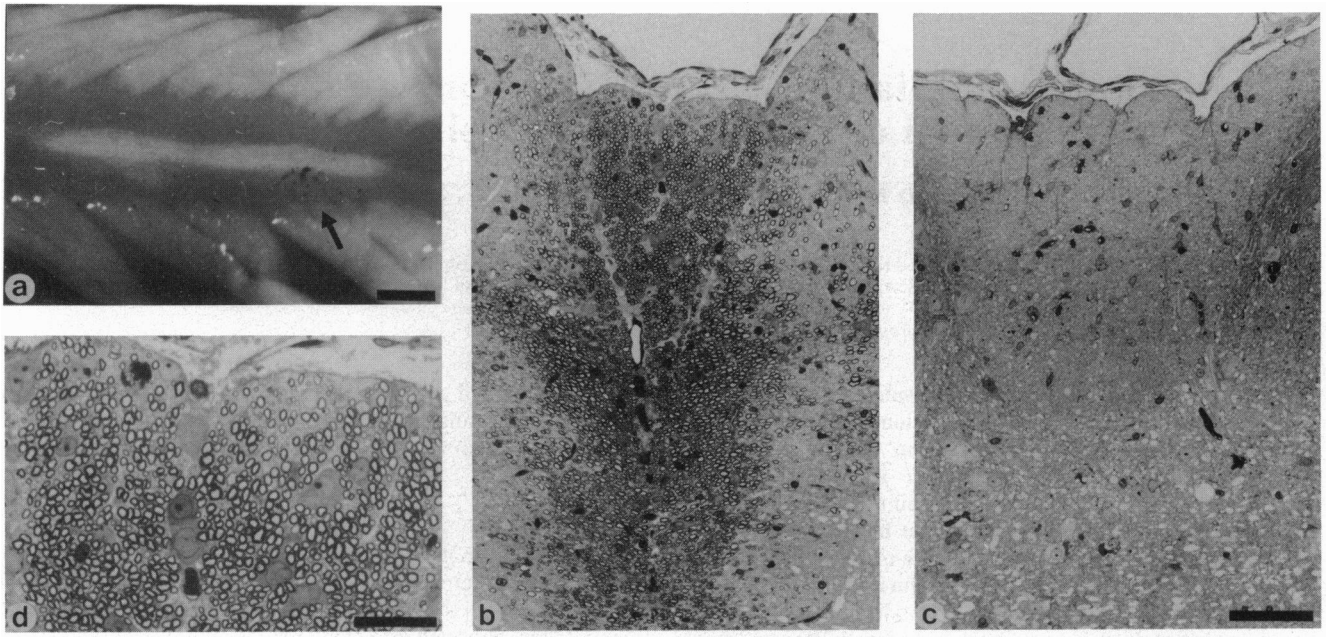

FIG. 1. (*a*) View of the site of transplantation (marked with charcoal; see arrow) showing an area of myelination by the transplanted cells. (*b*) A 1-μm section through the transplanted area shows that the white streak seen in *a* is a large patch of myelinated axons occupying most of the dorsal column. Numerous oligodendrocytes can be seen throughout the region. (*c*) No myelinated axons are present in an adjacent nontransplanted region. (*d*) In a higher power view of the region shown in *b*, details of the normal myelin formed by the transplanted glial cells can be seen. (Bars: *a*, 0.5 mm; *b* and *c*, 50 μm; *d*, 20 μm.)

were occasionally signal averaged (n = 8) when <1 mV but were often >1 mV, at which amplitudes little or no benefit in the signal/noise ratio was gained by signal averaging. All experiments reported here were performed at room temperature.

The conduction velocity of the compound field potential was determined as described (14). With the bipolar stimulus electrodes in a fixed position on the midline of the dorsal columns, the recording electrode was stepped at intervals of either 0.25 or 0.5 mm along the rostrocaudal axis of the dorsal columns and field potentials obtained at each position. When possible, the recording track was situated to include several field potentials from the transplant zone as well as the adjacent nontransplant zone within one rostrocaudal conduc-

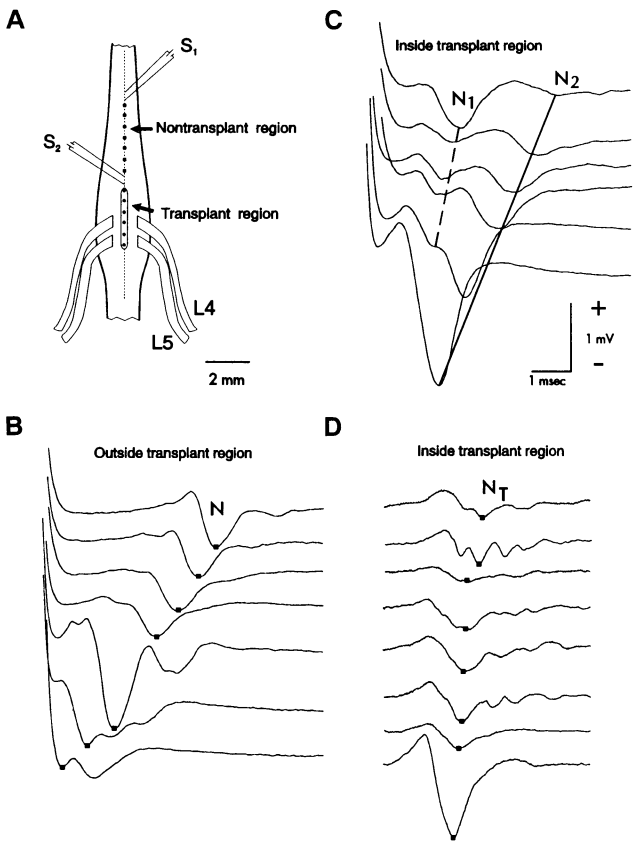

FIG. 2. Field potentials recorded from transplant and nontransplant regions of the dorsal columns. (*A*) A dimensionally accurate schematic of the transplanted md spinal cord showing the longitudinal extent of the transplant region (≈3 mm in this animal). Two separate stimulation sites are shown (S_1 and S_2), one for a recording track within the transplant region and another for a recording track outside the transplant region more rostrally. The recording interval is 0.5 mm for both of the tracks shown here. (*B*) Field potentials from dorsal columns outside the transplant region typically show a single main negativity with occasional components either before or after the main negativity (note the fourth trace from the top). (*C*) Field potentials from the transplant region of the same animal shown in *B* show two separate negativities (N_1 and N_2) with increasingly distinct latencies as the recording electrode is moved further away from the stimulus site. The stimulus site for this track is outside the transplant region, indicating propagation of the impulse across the amyelinated–myelinated junction. (*D*) Field potentials recorded at intervals of 0.25 mm from the transplant region of a different experimental animal show a single main negativity N_T without separately propagating components.

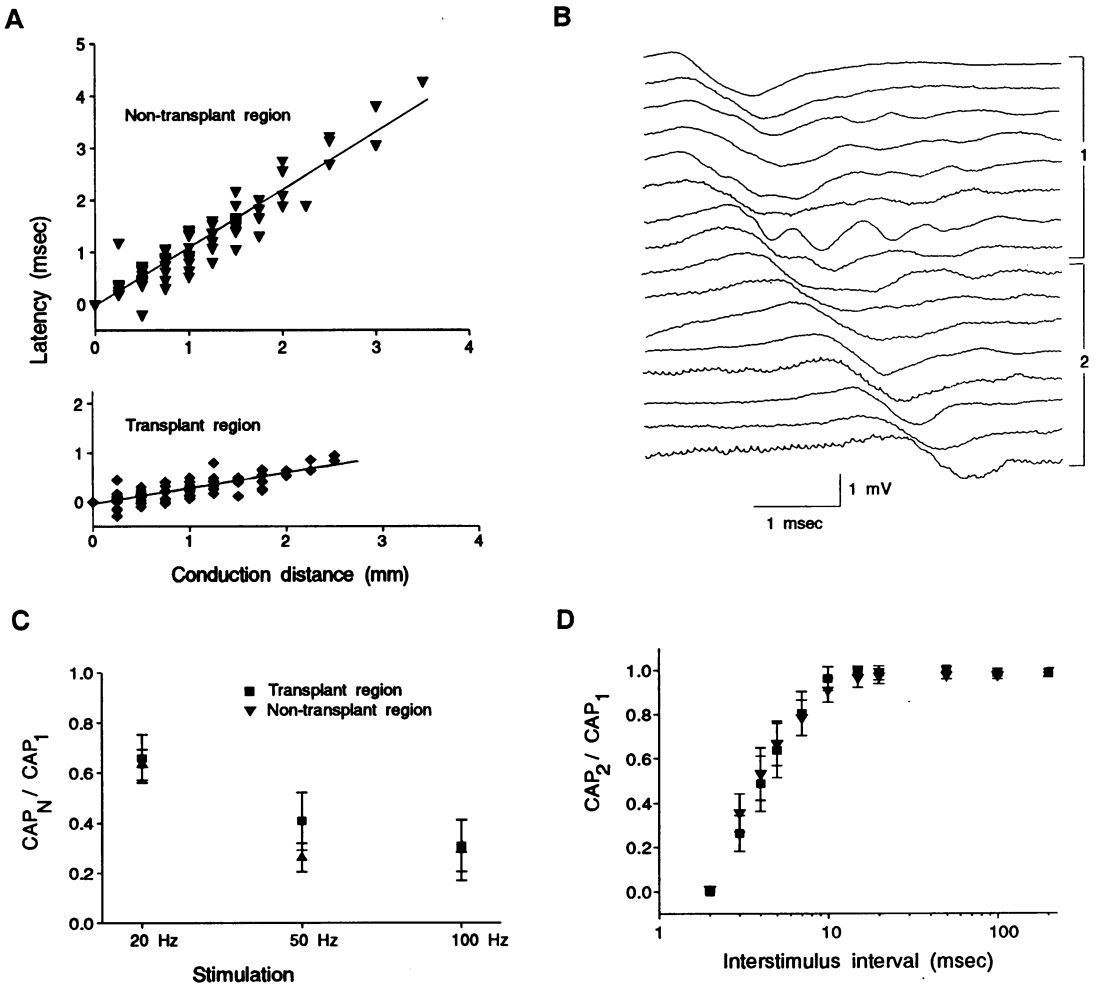

FIG. 3. Aggregate conduction latencies and refractory properties in transplant regions of the md dorsal columns. (*A*) (*Upper*) Latency of the main negativity from 100 recording sites from 17 recording tracks outside the transplant region of the dorsal columns. Latency of the main negativity from the closest recording site in each track has been set at 0 msec. Slope of the line of linear regression was 1.12 ± 0.04 msec/mm, yielding an average conduction velocity of 0.9 ± 0.03 m/sec (means ± SEM). (*Lower*) Similar aggregate data from the N_T and N_1 components from the transplant region show a significantly smaller increase in latency with increasing conduction distance, with an average conduction velocity of 3.2 ± 0.23 m/sec. (*B*) Field potentials obtained at equally spaced distances within (*1*) and outside (*2*) the transplant zone demonstrating the security of conduction into the transplant zone. (*C*) Ability of axons within the transplant zone to follow tetanic stimuli is similar to that of axons outside the transplant region. Shown here is the ratio of the amplitudes of the first and last compound action potentials (CAPs) for tetanic stimuli of 20 Hz (10 sec), 50 Hz (10 sec), and 100 Hz (2 sec). (*D*) Double-shock experiments showing the ratio of test CAP to control CAP for interstimulus intervals of 2–200 msec, showing that the time course of recovery for impulse conduction is similar inside and outside the transplant region.

tion track. The conduction velocity of the field potentials was calculated by obtaining the inverse of the slope of linear regression of the aggregate latency vs. distance data.

The preparation for single cell recording was similar to that for obtaining field potentials except that the L4 and L5 dorsal root ganglia were maintained in continuity with the spinal cord during the initial dissection. The ganglion was secured to the bottom of the recording chamber with four to six 0.1-mm steel pins and intracellular recordings were obtained with 100 MΩ glass microelectrodes filled with 4 M KOAc and 10 mM KCl.

RESULTS

In each of the 10 transplanted rats in this series there was an opaque white patch several millimeters long visible on the dorsal surface of the spinal cord. The dorsal columns of nontransplanted md rat spinal cords are translucent because they lack myelin. Examination of these patches with a dissecting microscope showed that they were continuous and limited in all but one case to the dorsal columns. Fig. 1*a* shows a transplanted spinal cord with a clearly visible patch 3.0 mm long. Most of the cross-sectional area of the dorsal columns had become myelinated after transplantation of glial cells, which appear numerous throughout the region of repair (Fig. 1 *b* and *d*). Rostral and caudal to these areas of myelination the dorsal columns contained very few or no myelinated axons as seen in nontransplanted md spinal cords (Fig. 1*c*).

Glass microelectrodes were used to record local field potentials from the transplant region and adjacent nontransplanted region of the first six rats in this series (Fig. 2*A*), and conduction properties of single axons were studied with intracellular recordings in an additional four rats. Fig. 2 shows field potentials obtained at rostrocaudal intervals of 0.5 mm from separate tracks in the nontransplant region (Fig. 2*B*) and adjacent transplant region (Fig. 2*C*) of the dorsal column. In this sequence of recordings within the transplant region (Fig. 2*C*), there are two negativities; the change in latency per increment of conduction distance is consistently smaller for the first negativity (N_1) than for the second, larger negativity (N_2). The change in latency for the first negativity

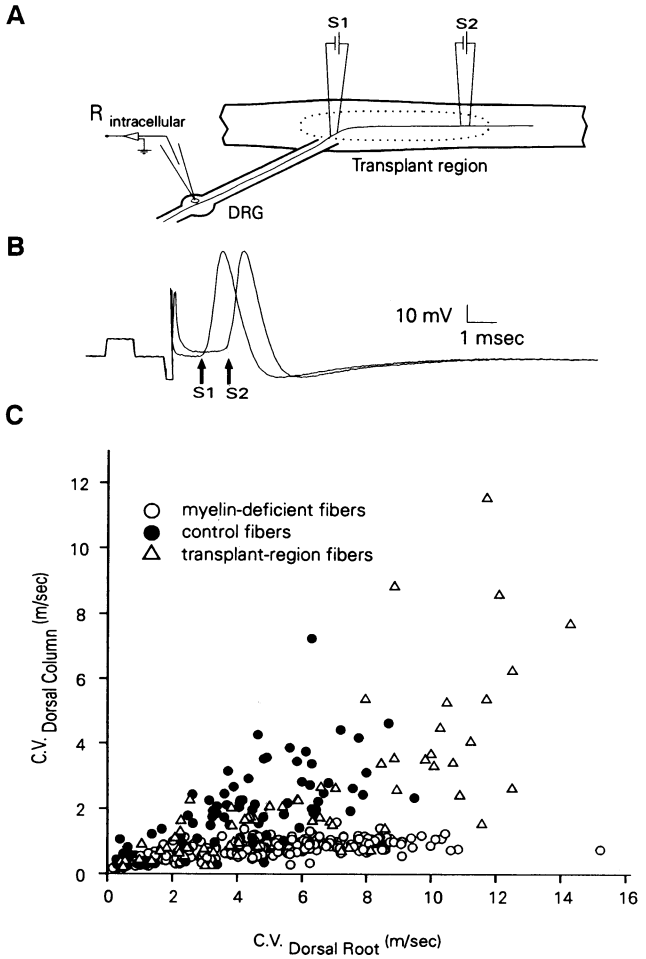

FIG. 4. Single-cell recording of impulse conduction through the transplant region. (*A*) Schematic showing placement of two stimulating electrodes in the transplant region and one intracellular recording electrode ($R_{intracellular}$) in the attached dorsal root ganglion (DRG). (*B*) Intracellularly recorded action potential of a dorsal root ganglion neuron stimulated at two different sites of the transplant region. Interstimulus distance is 2.0 mm and interstimulus latency shift is 0.77 msec, resulting in a dorsal column conduction velocity of 2.6 m/sec for this axon. (*C*) Aggregate data from dorsal column axons within the transplant region (△; $n = 67$), nontransplanted md (○; $n = 258$), and nontransplanted control rats (●; $n = 95$). C.V., conduction velocity.

from the transplant region is also significantly smaller than that of the main negativity recorded in the adjacent nontransplanted dorsal columns (Fig. 2*B*). In 6 of 11 of the conduction tracks obtained in the transplant region a separate conduction velocity was obtainable for N_1 and for the typically larger N_2. In the remaining five conduction tracks obtained from the transplant region there was only one repeatedly identifiable negativity (N_T; Fig. 2*D*), which was also the case for all 17 recording tracks obtained outside the transplant region. As shown in Fig. 2*B*, the dorsal column field potential from the nontransplanted region can occasionally have more than one component, but the second component could not be reliably identified throughout the conduction track, and when it could be seen in two or three consecutive field potentials it tended to have a latency shift equal to that of the main component.

To determine the conduction velocity of the axons the latency of the negativities was plotted against conduction distance. Fig. 3*A* shows the aggregate data from 17 tracks through nonmyelinated regions outside the transplanted area, which resulted in an average conduction velocity of 0.9 ± 0.03 m/sec (mean ± SEM; 100 latency points from 17 separate conduction tracks). The conduction velocity of the N_1 and N_T components from within the transplant region (3.2 ± 0.2 m/sec; mean ± SEM; 83 latency points from 11 separate conduction tracks; Fig. 3*A*) was ≈3 times faster than the conduction velocity from outside the transplant region. The conduction velocity of the N_2 component from within the transplant region (1.2 ± 0.07 m/sec; mean ± SEM; 53 points from 6 tracks) was only slightly faster than the conduction velocity from the nontransplant region.

The field potential could be observed to propagate either into or out of the transplant area in all six rats studied (Fig. 3*B*). In the four cases in which a conduction track encompassed both myelinated and nonmyelinated regions there was an approximate doubling of conduction velocity as the action potential propagated into the myelinated region. In addition, fibers within the transplant region displayed frequency–response properties virtually identical to fibers outside the transplant region (Fig. 3 *C* and *D*). Importantly, the ability of fibers within the transplant region to follow tetanic stimuli at test frequencies up to 100 Hz is equivalent to that outside the transplant region.

The conduction velocity of single axons in the transplant region was assessed by using intracellular recordings from single dorsal root ganglion neurons with antidromic stimulation of the dorsal columns (Fig. 4 *A* and *B*). Stimulating electrodes were placed at the dorsal root entry zone and at the rostral edge of the transplant region. Latency measurements from 67 axons conducting through the transplant region yielded an average conduction velocity that was ≈3 times faster than axons from nontransplanted md rats and comparable with axons from age-matched controls (transplanted rats, 2.3 ± 0.3 m/sec; $n = 67$; md rats, 0.76 ± 0.02 m/sec; $n = 258$; age-matched controls, 1.9 ± 0.13 m/sec; $n = 95$; means ± SEM). A plot of dorsal column conduction velocity vs. dorsal root conduction velocity for axons from each of the three experimental groups shows a majority of axons in the transplant region having a conduction velocity significantly greater than dorsal column axons of nontransplanted md rats (Fig. 4*C*). Propagation of the action potential evoked at the S2 electrode into the dorsal root ganglion also demonstrates that axonal conduction occurred through the zone of potential impedance mismatch from the transplant region to nontransplanted parts of the host nervous system.

DISCUSSION

The potential for improvement of conduction properties in demyelinated axons was suggested by physiological studies in the peripheral nervous system (15–17) and CNS (18–20), which demonstrated that remyelination by endogenous Schwann cells and oligodendrocytes leads to increased conduction velocity. The results reported here demonstrate that myelination of central axons by exogenous CNS glial cells leads to propagation of impulses with a significantly higher conduction velocity than in nontransplanted regions. Action potentials can be initiated outside the transplant region, propagate into the transplant area, and continue beyond the transplant region. Moreover, axons myelinated by the transplanted cells have refractory periods and frequency-following properties similar to those of axons in adjacent nontransplanted regions, which have been shown to have refractory and frequency-following properties similar to control myelinated fibers (14). These results demonstrate that myelination, as a result of transplantation of exogenous glial cells into amyelinated spinal cord, results in restoration of normal conduction properties.

Although detailed biophysical data are not yet available with respect to the transplanted md axons, it is likely that the increased conduction velocity is due to myelination. Computer simulation suggests that myelination, even with thin

myelin sheaths (7) or short myelinated internodes (8), should increase conduction velocity, assuming that relatively normal axonal membrane properties are established at the newly formed nodes of Ranvier. The conduction velocity of axons in the transplant region was increased ≈3-fold relative to the nontransplant region and was greater than the conduction velocity in normal CNS nonmyelinated axons (21); in the absence of myelination a significant increase in axon diameter would be required to account for this increased conduction velocity in the transplant zone. Saltatory conduction in normal myelinated axons requires a sodium channel density at the nodes of Ranvier that is much higher ($\approx 10^3$ per μm^2) than in normal nonmyelinated axons (9, 22). Although we have not as yet measured sodium channel densities at the newly formed nodes along transplanted axons, it is known that increased sodium channel densities develop in chronically demyelinated spinal cord axons (23), and that following remyelination by endogenous myelin-forming cells in peripheral nerve (24) and spinal cord (25), sodium channels are deposited at the newly formed nodes of Ranvier. In view of the increased conduction velocity and normal frequency-following properties in the transplant area, it is likely that transplantation of myelin-forming cells leads to formation of relatively mature nodes of Ranvier.

The timing of the transplants reported here was developmentally appropriate for myelination, allowing these experiments to focus on whether axons with myelin formed after transplantation can conduct impulses. The present study, however, was limited to studying myelin formed within only 2 weeks of transplantation. Future studies will examine the long-term effects of glial transplantation and the effects of glial transplantation into adult recipients (26) and will determine the optimal method of obtaining a pure population of myelin-forming precursor cells (4, 26).

In this study, we examined the effects of glial transplantation on conduction velocity. It would be interesting to determine whether conduction block is overcome by transplantation of glial cells, as has been demonstrated by agents that block potassium channels such as 4-aminopyridine (27). However, conduction block has not been observed in the md rat (14); future studies in other models will be needed to examine conduction block and safety factor. Our results clearly demonstrate that conduction velocity increased nearly 3-fold as a result of glial cell transplantation. Since there is a 4-fold reduction in conduction velocity in md spinal cord axons (14), the 3-fold increase in conduction velocity seen here demonstrates a significant return toward normal function in amyelinated CNS axons as a result of the transplantation of myelin-forming cells. This provides a demonstration that physiological properties, as well as anatomical properties, of pathological white matter tracts can be favorably altered by glial cell replacement.

This work was supported by the National Multiple Sclerosis Society (J.D.K. and S.G.W.), National Institutes of Health (I.D.D. and J.D.K.), the Myelin Project and Elisabeth Elser Doolittle Trust (I.D.D.), and the Allen Charitable Trust. D.A.U. was supported in part by an Eastern Paralyzed Veterans of America Multiple Sclerosis Fellowship and the Medical Scientist Training Program.

1. Duncan, I. D., Hammang, J. P. & Gilmore, S. A. (1988) *Glia* **1**, 233–239.
2. Duncan, I. D., Hammang, J. P., Jackson, K. E., Wood, P. M., Bunge, R. P. & Langford, L. (1988) *J. Neurocytol.* **17**, 351–360.
3. Rosenbluth, J., Hasegawa, M., Shirasaki, N., Rosen, C. L. & Liu, Z. (1990) *J. Neurocytol.* **19**, 718–730.
4. Duncan, I. D., Paino, C., Archer, D. R. & Wood, P. M. (1992) *Dev. Neurosci.* **14**, 114–122.
5. Lachapelle, F., Lapie, P. & Gumpel, M. (1992) *Dev. Neurosci.* **14**, 105–113.
6. Blakemore, W. F. (1977) *Nature (London)* **266**, 68–69.
7. Koles, Z. J. & Rasminsky, M. (1972) *J. Physiol. (London)* **227**, 351–364.
8. Brill, M. H., Waxman, S. G., Moore, J. W. & Joyner, R. W. (1977) *J. Neurol. Neurosurg. Psych.* **40**, 769–774.
9. Moore, J. W., Joyner, R. W., Brill, M. H., Waxman, S. G. & Najar-Joa, M. (1978) *Biophys. J.* **21**, 147–161.
10. Sears, T. A., Bostock, H. & Sherratt, M. (1978) *Neurology* **28**, 21–26.
11. Waxman, S. G. & Brill, M. H. (1978) *J. Neurol. Neurosurg. Psych.* **41**, 408–417.
12. Hirano, A. & Dembitzer, H. M. (1978) in *Physiology and Pathobiology of Axons*, ed. Waxman, S. G. (Raven, New York), pp. 68–82.
13. Funch, P. G. & Faber, D. S. (1984) *Science* **225**, 538–540.
14. Utzschneider, D., Black, J. A. & Kocsis, J. D. (1992) *Neuroscience* **49**, 221–228.
15. Pender, M. P. (1989) *Brain* **112**, 393–416.
16. Shrager, P. (1988) *J. Physiol. (London)* **404**, 695–712.
17. Smith, K. J., Bostock, H. & Hall, S. M. (1982) *J. Neurol. Sci.* **54**, 13–31.
18. Smith, K. J., Blakemore, W. F. & McDonald, W. I. (1981) *Brain* **104**, 383–404.
19. Blight, A. R. & Young, W. (1989) *J. Neurol. Sci.* **91**, 15–34.
20. Felts, P. A. & Smith, K. J. (1992) *Brain Res.* **574**, 178–192.
21. Waxman, S. G. & Swadlow, H. A. (1977) *Prog. Neurobiol.* **8**, 297–325.
22. Ritchie, J. M. & Rogart, R. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 211–215.
23. Black, J. A., Felts, P., Smith, K. J., Kocsis, J. D. & Waxman, S. G. (1991) *Brain Res.* **544**, 59–60.
24. Ritchie, J. M., Rang, H. P. & Pellegrino, R. (1981) *Nature (London)* **294**, 257–259.
25. Weiner, L. P., Waxman, S. G., Stohlman, S. A. & Kwan, A. (1980) *Ann. Neurol.* **8**, 580–583.
26. Groves, A. K., Barnett, S. C., Franklin, R. J. M., Crang, A. J., Mayer, M., Blakemore, W. F. & Noble, M. (1993) *Nature (London)* **362**, 453–455.
27. Targ, E. F. & Kocsis, J. D. (1985) *Brain Res.* **328**, 358–361.