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Research Article

Transplantation of Genetically Modified Fibroblasts Expressing BDNF in Adult Rats with a Subtotal Hemisection Improves Specific Motor and Sensory Functions

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Objective: We have previously reported that grafting fibroblasts genetically modified to express brain-derived neurotrophic factor (BDNF) into a subtotal cervical hemisection site that destroys the entire lateral funiculus will promote regeneration of rubrospinal axons and growth of other axons, prevent atrophy and death of axotomized red nucleus neurons, and improve forelimb use during spontaneous vertical exploration. We have now extended these studies by using additional sensorimotor tests to examine recovery. **Methods:** The range of tests used included those in which the intervention did not improve recovery, those in which the intervention was associated with recovery, and those that showed little deficit. The selected tasks tested both sensory and motor functions and both forelimb and forelimb function. We used the open-field locomotor rating scale (BBB), locomotion on a narrow beam, forelimb use during swimming, horizontal rope walking, and a somatosensory asymmetry (patch-removal) test. After testing during an 8-week recovery period, a second lesion was made just rostral to the initial lesion/transplant site to test the role of the transplant in recovery. The rats were then retested for a further 5 weeks after the repeated lesion. **Results:** The horizontal rope, swim, and patch-removal tests were reliably sensitive to the subtotal hemisection injury. Fb/BDNF-transplanted animals recovered motor functions on the horizontal rope-crossing test, and this recovery was abolished by a second lesion just rostral to the first lesion/transplant. In the patch-removal test, the latency to contact the affected limb was shorter in Fb/BDNF-treated rats than in the control group, and this effect was completely abolished by a second lesion. **Conclusions:** The rope-crossing and patch-removal tests are particularly useful tasks for assessing the beneficial effects of BDNF-expressing grafts in this injury model. **Key Words:** BDNF—Fibroblasts—Transplants—Recovery of function.

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Modern therapeutic strategies are aimed at improving recovery after lesions to the central nervous system (CNS). The ex vivo gene therapy approach is a convenient way to deliver therapeutic molecules that modify the CNS to create an environment more conducive to regeneration and ultimately to functional recovery. Intraspinal transplants of genetically modified fibroblasts expressing NT3 have been shown to promote limited corticospinal tract regeneration and partial improvement in limb-placing reactions in a grid walking test (1). We have reported that axons in another supraspinal system, the rubrospinal tract, regenerate at least three to four segments caudal to a lesion of the lateral funiculus in cervical spinal cord when fibroblasts genetically modified to produce brain-derived neurotrophic factor (BDNF) were grafted into the injury site (2). Growth of descending serotonergic and calcitonin gene–related peptide (CGRP)-labeled dorsal root axons also was observed in this model. In addition, these grafts rescued axotomized red nucleus neurons destined to die or atrophy below the level of resolution (2a). Finally, this procedure led to limited recovery of a forelimb function, as assessed by a test that evaluated use of the forelimbs for weight-bearing and weight-shifting movements during exploration of vertical surfaces in a cylindrical chamber (2, 3).

The goals of this study were to extend our previous work by examining the deficits and spontaneous recovery on several additional behavioral tests after an injury that destroyed the lateral funiculus but spared the corticospinal tract and then to determine whether grafting of BDNF-expressing fibroblasts can reverse or ameliorate some of the deficits. We used tests that focus on both forelimb and hindlimb functions as well as on sensorimotor integration. At the end of the recovery period, we performed a second subtotal hemisection just rostral to the initial lesion to determine whether the recovery in transplant recipients would be abolished by the second injury and thus be attributable to the presence of the transplant.

Methods

Surgery

Detailed surgical procedures are described elsewhere (2). In brief, after an intraperitoneal injection of acepromazine maleate (0.7 mg/kg; Fementa Animal Health Co., Kansas City, MO, U.S.A.), ketamine (95 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA, U.S.A.), and rompun (10 mg/kg, Bayer Co., Shawnee Mission, KS, U.S.A.), a laminectomy was made at the C3–4 level. The spinal cord was then exposed, and the dura cut. A subtotal hemisection was created by making a shallow incision in the right side of the dorsal spinal cord and then aspirating the entire lateral funiculus. The dorsal funiculus, most of the gray matter, and most of the ventral funiculus were spared. Eighteen rats were prepared and randomly divided into three groups: subtotal hemisection alone (Hx; n = 6), subtotal hemisection + fibroblasts modified to express BDNF (Fb/BDNF; n = 6), and subtotal hemisection + unmodified Fb (Fb; n = 6). A piece of absorbable gelatin sponge (Gelfoam) soaked with Fb/BDNF in growth medium, unmodified Fb cells in growth medium, or a piece of Gelfoam soaked in growth medium without cells was placed into the lesion site. Another 10 μl of Fb/BDNF cells or Fb (10^6 cells) was injected into the Gelfoam containing Fb/BDNF or Fb. These genetically modified fibroblasts have been shown to secrete bioactive BDNF for ≥1 month (2, 4). The dura was sutured with 10-0 silk sutures, and the muscle and skin were closed in layers. At the completion of the surgery, all animals received a single bolus intravenous injection of methylprednisolone (30 mg/kg; Pharmacia and Upjohn Company, Kalamazoo, MI, U.S.A.) through the tail vein. Animals were kept on a heating pad, observed closely until awake, and then returned to home cages. Before and after the initial lesion, all rats were immunosuppressed with cyclosporin A (CsA; Sandoz Pharmaceuticals, East Hanover, NJ, U.S.A.). Rats were given daily CsA (1 mg/100 g b.w.) subcutaneously 3–5 days before the transplantation procedures and continued for 2 weeks after surgery. At 3 weeks, rats were switched to oral CsA (50 μg/ml) in the drinking water for the duration of the experiment.

At the end of the initial testing period (8 weeks), rats received a second laminectomy at C2–3, and the right lateral funiculus was removed with the same procedure as described for the initial lesion. The second lesion was guided visually, and placed as close to the initial lesion/transplant site as was practical. The animals were tested for an additional 5 weeks to examine deficits and recovery from the second lesion and then killed. The second lesion axotomizes all axons that have grown into the rostral border of the graft and thus tests the role of these axons in the recovery. The proximity of the two lesions prevented differentiating the extent of the first and second lesions.

Functional Tests

On arrival at our animal facility, rats were allowed to acclimate to the new environment for ≥48 h. Rats were removed from the home cage and gently stroked and handled for 5 min daily for several days. Baseline performance was measured before surgery. Rats were tested weekly starting 1 (patch-removal test) or 2 (rope-crossing and swim tests) weeks postoperatively. Testing sessions were video-recorded, except for the patch-removal test, and scored later by observers who were uninformed as to the surgical interventions the animal received. The BBB and narrow-beam tests were administered at 4 weeks after surgery. The following behavioral tests were used:

Horizontal Rope-Crossing Test. Animals were trained to traverse a rope measuring 4.4 cm in diameter and 83 cm in length. The rope was stretched horizontally rather than vertically, as in Thallmair et al. (5). The rope was elevated 30 cm from the table, and the ends of the rope were secured to posts. For each trial, the rope was pulled parallel to the floor to maintain consistent tension, but because the rope was suspended, the weight of the animal caused the rope to sag slightly during the crossing. Hindlimb weight support, precise foot placement, and adaptable balance are required to complete horizontal rope-crossing successfully. Rats were given a few conditioning trials and then tested for another five consecutive trials. Uninjured control animals cross the rope with few slips and no falls, and complete the task quickly. Behaviors were scored using both quantitative and rating methods. Quantitation included counting slips and falls during the rope crossing. Falls from the rope were scored as two slips (simultaneous slips by both hindlimbs). The numbers of slips and falls were then added to constitute an *error score*, the total number of errors per 10 steps. For the ranking method, a *deficit score* was calculated. Two or three observers viewed the videotapes of rats traversing the rope and scored the quality of locomotion based on a 4-point rating system (0, normal locomotion; 1, good, close to normal locomotion; 2, moderate with some obvious deficits such as inability to maintain consistent placement of the limbs under the body for weight support during the traverse; and 3, poor performance with great difficulty in crossing and/or frequent falls and slips). The following three features were evaluated with the 4-point rating system: fore- and hindlimb placement; hindquarter weight support; and posture. An animal receiving a score of 9 had the greatest deficits. Rats that failed to cross the rope because of an inability to maintain the hindlimbs on the rope were assigned the maximal deficit score of 9. To minimize potential bias in scoring, each rat was observed 3 times with $\geq$2 days between observations. The final deficit score was the mean of the three independent observations. Inter-rater reliability was 0.98.

Swim Test. This task can be used to examine both forelimb and hindlimb movements in a locomotor task that does not require weight support (6). In this study, we focused on the forelimb function. Animals were placed in a tank of water (water temperature, 20ºC) measuring 150L × 37W × 40H cm and trained to swim toward a platform where its cagemates were placed. When swimming in a straight line, intact rats swim with the forelimbs adducted underneath the chin and held immobile (planing behavior), and the rat is propelled forward by alternating hindlimb stroking movements (7). The swim test was videotaped, and the presence or absence of forelimb planing behavior and hindlimb stroking movements was noted for each rat. In addition, the angle of each forelimb with the body was measured, and the difference in angle between unimpaired and impaired forelimbs was calculated to identify deficits in forelimb posture.

Patch-Removal Test. The patch-removal task assesses the competency of somatosensorimotor function because the patch must first be detected and then removed. A small round patch of adhesive label (1.2 cm in diameter, Avery International) was placed on the distal radial aspect of both forelimbs, one at a time. The order in which the patches were applied to each forelimb was random, and both limbs were touched briefly by the experimenter at the same time just before placing the animal back into its home cage. The adhesivity of the patches was such that they did not fall off spontaneously and hair did not stick to the patch when it was removed; any trial in which the patch was not fully adherent to the forelimb was not scored. Timing began when the home-cage door was closed. The order and latency of contact and removal of the patch were recorded for each of four trials. Each trial was terminated when the animal removed the patch, or after 120 s. Rats contacted the patches with their mouths and removed them by grasping with their teeth and pulling upward slightly from the forelimb; thus the patch-removal test did not directly assess forelimb motor function.

BBB Open-Field Locomotor Test. Rats were placed individually in an open-field enclosure for 5 min and scored by at least two investigators according to the BBB rating scale (8). BBB rating ranges from 0 (no observable hindlimb movements) to 21 (normal locomotion). Normal rats consistently receive BBB scores of 21.

Narrow-Beam Test. Efficient locomotion on an elevated narrow beam requires that animals use careful foot placement and weight support with fore- and hindlimbs (9, 10). Normal rats cross the beam quickly with few errors. Operated and control animals were trained to walk on an elevated (100 cm) narrow runway (80 × 5 cm), and the number of foot slips while walking on the beam was recorded.

Statistical Analysis

All data were analyzed with parametric and nonparametric tests. The BBB scores and scores from the horizontal rope test were analyzed nonparametrically. The remaining data were analyzed with a two-way mixed analysis of variance (ANOVA) (Treatment × Weeks) and followed by a one-way ANOVA when appropriate.

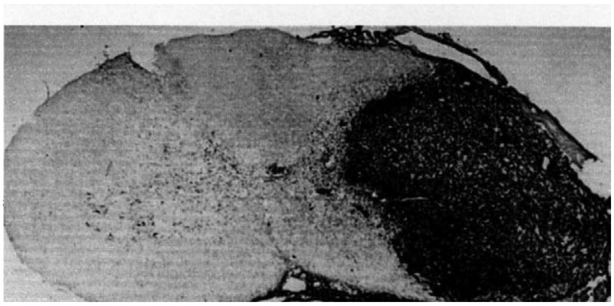

Figure 1. Photomicrograph of cross section through spinal cord showing a graft of fibroblasts genetically modified to secrete BDNF in the site of a subtotal hemisection at C3. Note that the entire dorsolateral funiculus is lesioned, thus ablating the rubrospinal, dorsal spinocerebellar, and Lissauer's tracts. The graft fills the lesion site without cyst formation.

The Fisher's LSD test was used for post hoc multiple comparisons when necessary (Statview; Abacus Concepts, Inc., Berkeley, CA, U.S.A.).

Results

Histology

The lesion ablated the dorsal lateral quadrant of the spinal cord, including the area occupied by the rubrospinal, dorsal spinocerebellar, and Lissauer's tracts. The Fb and Fb/BDNF grafts contained healthy cells, which maintained the shape of the spinal cord (Fig. 1). The lesions and transplants were similar to those described in Liu et al. (2).

Horizontal Rope-Crossing Test

Unoperated control animals make few slips and no falls on this test (0.62 ± 0.01 errors/10 steps). All operated animals showed deficits, but the deficits were modest in the Fb/BDNF transplant recipients. A two-way ANOVA (Treatment × Time) showed no significant interaction effects ($p = 0.27$), but a significant main effect of treatment ($p < 0.05$) was observed, indicating that Fb/BDNF-treated animals slipped or fell from the rope less frequently than those treated with unmodified fibroblasts or subtotal hemisection. The animals' performances were stable, regardless of the treatment received, over the 8-week period (Fig. 2), indicating that recovery occurred primarily during the first 2 weeks. At all time points, the Fb/BDNF group had lower error scores, and thus better performance compared with the operated control groups in this task. In addition to the fewer slips and falls, other aspects of their performance on rope walking (including limb placements, hindquarter weight support, and posture) were superior in the Fb/BDNF group (Fig. 3). These features were reflected in significantly lower deficit scores. The deficit score may be a more sensitive measure than the error score because it indicates that recipients of unmodified fibroblasts perform better than the lesion-alone group, although significantly less well than the Fb/BDNF group (Fig. 4).

After the second lesion (Figs. 2 and 4), the operated control groups showed little additional deficit. The Fb/BDNF group made significantly more foot slips and falls from the rope (errors) after the repeated lesion and was also significantly more impaired than either the unmodified Fb or hemisection groups ($p < 0.05$), suggesting a greater vulnerability to adverse repeated lesion

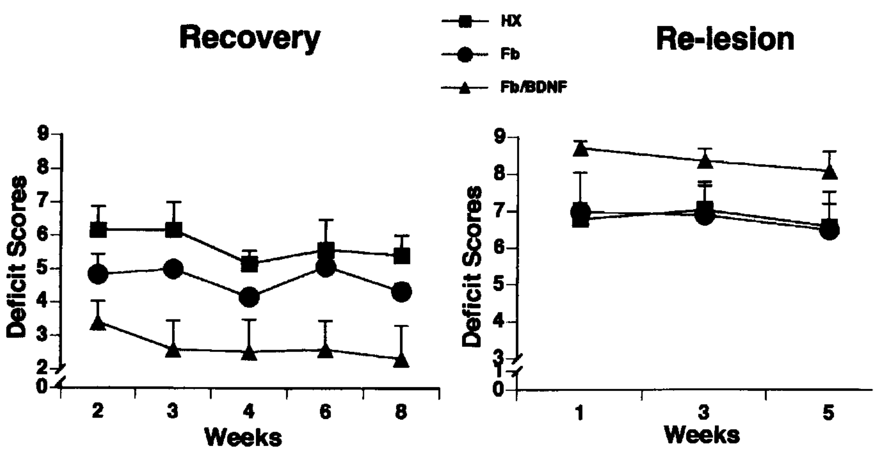

Figure 2. Horizontal-rope test. **a:** Time course of recovery. Deficit score 2–8 weeks postoperatively on the rope task in rats receiving Fb/BDNF transplants (triangles), unmodified fibroblasts (circles), and lesion only (squares). Rats in the Fb/BDNF group had a significantly lower deficit score than those in the other two groups throughout the recovery period ($p < 0.05$). There was little improvement over the test duration in any group. Data expressed as mean ± standard error. HX, lesion-only operated controls; Fb, unmodified fibroblast transplant recipient, operated controls; Fb/BDNF, modified fibroblast transplant recipients. **b:** Effect of relesion. The Fb and HX groups showed no significant change in deficit score, but the deficit scores for the Fb/BDNF group were significantly greater than those before the second lesion and also significantly ($p < 0.05$) greater than those of the operated control groups after the second lesion.

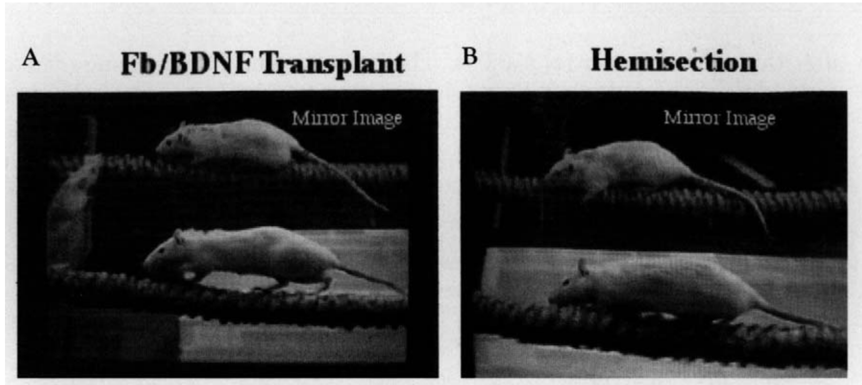

Figure 3. Rats crossing a rope 1 month after subtotal hemisection. A mirror placed behind the rat allows visualization of contralateral limbs during the crossing. **a:** A rat that received a transplant of BDNF-secreting fibroblasts. Note support of hindquarters with good trunk elevation and placement of paws under the body. **b:** A rat that received a lesion only. Note poor hindquarter support, posture, and limb, particularly forelimb, placement.

events. A similar pattern of results was observed for the deficit scores. Thus, these results showed that the second lesion abolished the treatment effect, and the Fb/BDNF group became significantly more impaired than the operated control groups. These effects persisted through the 5 weeks after surgery.

Swim Test

Unoperated control rats maintain both forelimbs in a planing position while swimming; there was no difference between the angles assumed by right and left forelimbs. Rats from all treatment groups used only the unimpaired forelimb to plane and showed no recovery of use of the impaired forelimb during the 8-week observation period (Fig. 5). The impaired forelimb usually remained perpendicular to the body axis. In animals with the most severe deficits the impaired limb was flexed as much as 180 degrees from the normal position and thus held nearly parallel to the body but directed caudally instead of rostrally. There was a tendency for the Fb/BDNF treatment to produce less deviation in the impaired limb position during planing than those treated only with unmodified Fb or hemisection-alone groups, although the differences among the groups were not statistically significant.

After the second lesion, there was a tendency toward greater impairment on the injured side of the body. In all groups, the injured forelimb was flexed ~150 degrees caudally from the unimpaired forelimb position. In the Fb/BDNF group, the impairment was significantly greater

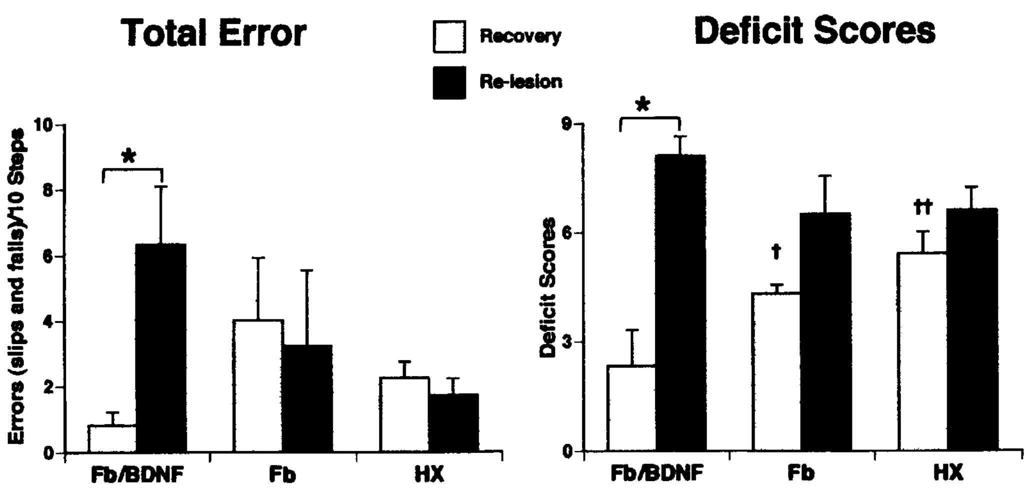

Figure 4. Rope-crossing test. Comparison of (**a**) error and (**b**) deficit score to assess the horizontal-rope test during recovery (open bars) and after repeated lesion (solid bars). Both measures show that Fb/BDNF recipients locomote significantly better on the rope test than do the operated-on control groups ($p < 0.05$). The FB/BDNF group shows fewer errors during the recovery period but a greater number of errors after the second lesion (*$p < 0.05$). The deficit score reveals significant differences among all three groups during the recovery period, with Fb/BDNF showing greatest improvement, and the lesion-only group, the greatest deficit. (ψ, Fb group is significantly more impaired than the Fb/BDNF group; $\psi\psi$, the Hx group is significantly more impaired than both the Fb/BDNF and the Fb groups). Data expressed as mean ± standard error of the mean.

after the second lesion ($p < 0.05$). There was no improvement in this behavior during the 5 weeks after the second lesion in any group.

Despite the lack of normal planing behavior by the impaired forelimb, all animals were able to use their hindlimbs to propel themselves toward the platform, and they used both fore and hindlimbs together to climb onto the platform.

Patch-Removal Test

Unoperated control animals quickly removed the patch from their forepaws with similar latencies for each limb and with no preference for right or left limb. All operated animals contacted the unimpaired forelimb first, and latencies to contact and remove the stimulus (patch) were significantly shorter for the unimpaired limb than for the impaired forelimb (Fig. 6). Very few rats in the Fb/BDNF group failed to contact and remove the stimulus from the impaired limb, whereas more rats with transplants of unmodified fibroblasts failed either to contact or remove the patch. The Fb/BDNF-treated group showed the shortest latencies to contact the stimulus on the impaired limb ($p < 0.05$). These effects were consistent up to 8 weeks (data not shown).

Very few animals in any group contacted the impaired limb after the second lesion. The Fb/BDNF group was significantly more impaired ($p < 0.05$) after the second lesion.

BBB and Narrow-Beam Tests

Neither BBB open-field locomotor ratings (Fig. 7) nor number of footslips/falls during the narrow-beam crossing (data not shown) showed any group differences at 4 weeks after surgery. Most rats that received unmodified fibroblasts or lesion-only rats were impaired in right forelimb function. For example, they flexed the forepaw during bouts of immobility in which the forelimbs were raised off the horizontal surfaces, but this did not interfere with overground locomotion in the open-field test or walking on the beam. Animals from all conditions crossed the narrow beam with few slips.

Discussion

We show that a battery of sensorimotor tests is needed to provide a comprehensive description of functional outcome after spinal injury and of the effects of tissue-grafting procedures. The rope-crossing test is sensitive not only to subtotal cervical spinal cord hemisection, but also to the effects of grafting BDNF-expressing fibroblasts into the injured region. Thus, both the rope test, which assesses a combination of hindlimb and forelimb motor capacity, and the cylinder test described previously (2), which measures spontaneous forelimb use, were useful in detecting improvements in motor function in animals with Fb/BDNF grafts and reversal of the improved function after repeated lesion rostral to the graft. Rats with Fb/BDNF grafts were also more successful at locating an adhesive patch placed on the affected forelimb, a sensory function, than were the operated control rats. The second lesion worsened performance only in the Fb/BDNF group. In vitro tests have shown that the genetically modified fibroblasts secrete bioactive BDNF (2), even 1 month after transplantation (4). The data are thus consistent with the conclusion that grafting cells genetically modified to produce neurotrophic factors into in-

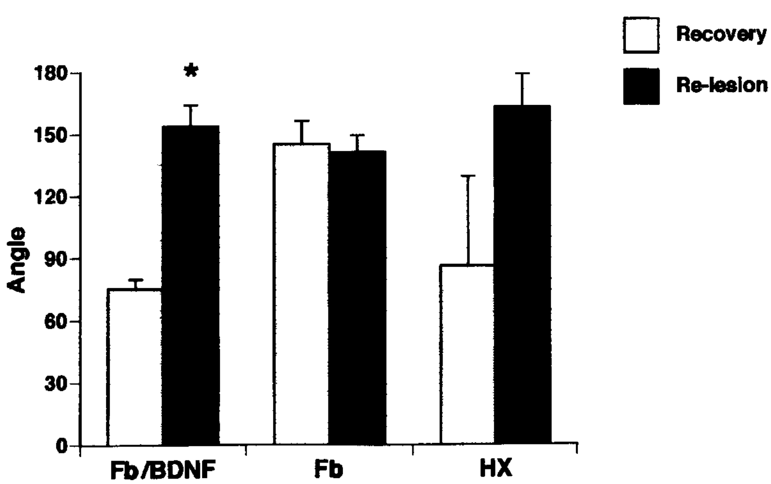

Figure 5. Swim test. Histograms showing the angle difference between the unimpaired and impaired forelimbs during planing in the recovery period (open bars) and after the repeated lesion (solid bars). None of the animals used the impaired limb to plane. Although there were no significant differences among the groups, the FB/BDNF group showed a significantly greater angle difference after the second injury than after recovery from the first injury ($p < 0.05$). Data expressed as mean ± standard error of the mean.

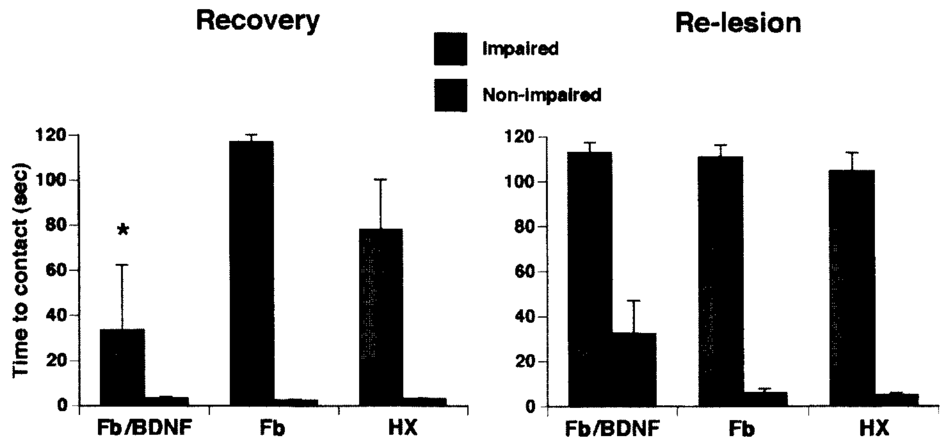

Figure 6. Patch-removal test. **a:** The latency to contact the patch is shown for each forelimb for the three groups. The unimpaired limb (solid bars) contacted the patch equally quickly in all three groups. The latency to contact with the impaired (gray bars) limb was much greater in all three groups. The latency to contact the impaired limb in the Fb/BDNF groups was significantly less than the latencies for the operated control groups ($p < 0.05$). Data expressed as mean ± standard error of the mean. **b:** After the second lesion, most animals did not contact the patch with the impaired limb during the test period, whereas there was little change in latency with the unimpaired limb. Data expressed as mean ± standard error of the mean.

jured spinal cord can improve both triggered and spontaneous motor function and sensory function.

The horizontal rope-crossing task showed persistent group differences during the 8-week postsurgery period; Fb/BDNF-treated rats had lower overall deficit scores, thus demonstrating better recovery. The better performance by the Fb/BDNF group was evident starting the second week after surgery, compared with other groups, and these group effects remained more or less constant throughout the testing period. On the rope, the animal is motivated to improve performance to prevent falling, and may quickly adopt alternative strategies using both the impaired and nonimpaired limbs. To traverse the rope successfully, the hindpaw digits must spread over the surface of the rope, which requires control of the digits and weight support in the hindlimbs. In addition, rats with an obvious forelimb impairment were able traverse the rope quite successfully. Rope crossing may therefore be more dependent on hindlimb function. The success in the rope-crossing task in all groups, despite the deficits, is thus likely to be due to the greater contribution of the hindlimbs, which are less impaired by the cervical lesion. The greater recovery in the Fb/BDNF group may reflect the additional effect of improved forelimb function.

The patch-removal task also was sensitive to deficits in this model of cervical spinal injury and to recovery

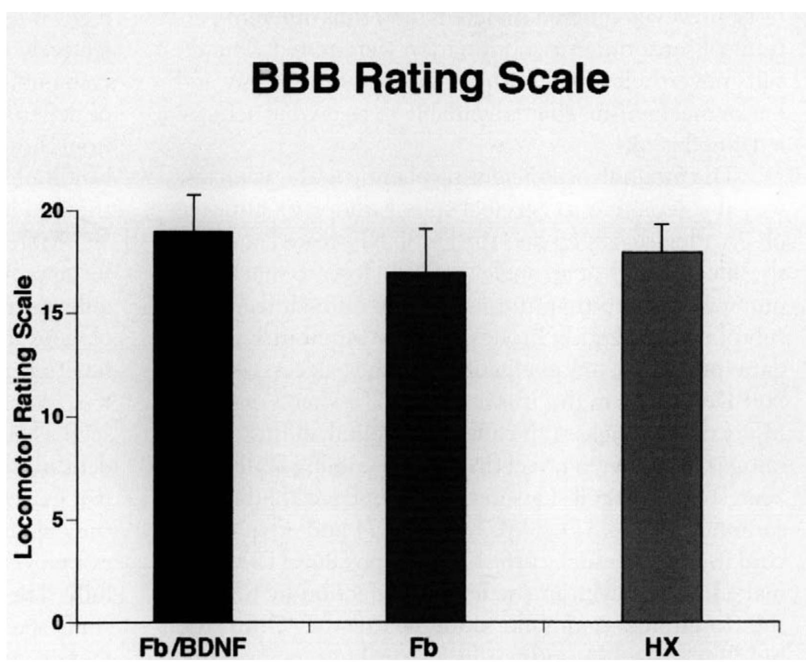

Figure 7. BBB scores at 4 weeks after surgery. All operated rats showed good spontaneous recovery and did not differ significantly from normal. Data expressed as mean ± standard error of the mean.

mediated by the Fb/BDNF transplants. The operated control rats frequently did not respond to the presence of the patch on the affected limb during the 2-min testing period. The Fb/BDNF rats recognized and removed the patch, although with a longer than normal latency, and this effect was abolished by the second lesion. Visual cues could also elicit a removal response but would presumably not be sensitive to spinal injury. These data suggest a recovery of somatosensory function in the Fb/BDNF rats.

The variant of the patch test that we used has been shown to be sensitive to interventions after other CNS injuries (11–14; see ref. 3 for review). Other modifications of the patch-removal test have been used after lesions of spinal systems to examine sensorimotor integration. Treatment with an antibody (IN-1) that neutralizes the myelin-associated inhibitory proteins improved performance in removal of a patch placed on the palmar surface of the forepaw in corticospinal tract–lesioned rats (5). Yet another variant in which adhesive patches are placed on the vibrissae or other parts of the snout (11) was used to test recovery in adult rats after neonatal cervical spinal injury (overhemisection), with and without transplants of fetal tissue (15). In that study, an adhesive patch was placed on the bridge of the rat's nose, and time and quality of the movements used to remove the patch were measured. Postural as well as forelimb grasping were impaired in both hemisected and transplanted rats, although transplanted rats performed better. These experiments suggest that greater recovery occurs after injury and transplantation in neonates. Thus, the patch-removal tests reveal deficits after quite different lesions and recovery after quite different interventions. Each of these tests, however, differed in details, and thus different patterns of sensorimotor coordination were tested. The results nevertheless reflect the redundancy in sensory and motor mechanisms that is available to be used to achieve a defined goal.

The forelimb posture during planing in the swim test was also sensitive to cervical spinal injury. Qualitative observation suggested that the Fb/BDNF-treated rats held the affected limb at an angle that was closer to that of the unimpaired limb than did rats in the unmodified Fb or subtotal hemisection groups, but the variability in the data precludes an evaluation of the success of the Fb/BDNF grafts in this injury model. The effects of spinal injury on the angle of the affected forelimb during swimming is, however, a novel finding. Swimming ability has been studied after lesions in the forelimb area of the sensorimotor cortex (FL-SMC) in rats (7) and after spinal cord injuries in chicks (16, 17) and opossums (18). Normal adult rats swim in the forward direction by holding the forelimbs immobile underneath the chin; the hindlimbs are used primarily for stroking movements. Paddling by the forelimbs may occur during turning, but when the animal is trained to swim quickly and in a straight line to a platform, its forelimbs do not move (7). The forward angle of the forelimbs presumably permits the animal to swim faster through the water. The spinal cord lesion did not disrupt the immobility in the affected forelimb but instead caused it to flex downward while swimming, or even backward toward the ventral surface of the trunk. This motor effect is quite different from that after unilateral damage to the motor cortex in which the animal paddles with the affected forelimb while the unaffected forelimb remains immobile in the normal planing posture, suggesting that there is a hemisphere specific loss of inhibitory cortical control (7). These results are interesting in light of the fact that injury usually produces a nonmovement and not inhibition of nonmovement. In the present study, none of the animals, regardless of the treatment, was able to plane with the affected forelimb in the normal posture.

Saunders et al. (18) measured the time to swim across a tank by opossums whose spinal cords were transected by crushing at the upper thoracic level as neonates. Opossums normally use a strategy for swimming that differs from that in the rat: both forelimbs and hindlimbs paddle. Many adult opossums whose spinal cords were crushed as neonates showed little difference from uninjured controls in the time to swim across the tank. The lack of impairment is likely due to both the regeneration and growth of new axons that occurs after neonatal injury in opossums and to the greater dependence on forelimb function, which is relatively unaffected by a thoracic lesion. Opossums with the more severe cut lesions do not recover use of their hindlimbs but nevertheless swim effectively using only their forelimbs (Saunders, personal communication). Hemisected chicks also show patterns of deficits and recovery of swimming that are different from those of rats (17). After a hemisection, the affected hindlimb showed a limited range of motion that contributed little to forward movement.

More subtle deficits were seen in the BBB open-field locomotor and narrow-beam tests. The operated control animals (unmodified Fb or subtotal hemisection) showed obvious motor deficits in the affected forelimb, but dysfunction in the home cage outside of a testing situation was less easily recognized. In the open-field test, deficits such as lack of weight support by the forelimb were evident in the affected forelimb but did not hinder the animal in overground locomotion. In the narrow-beam test, the deficits in the hindlimb were more obvious, but did not prevent the animals from crossing the beam successfully. The BBB locomotor rating scale was developed to study specifically hindlimb deficits in overground locomotion after contusion injury. Because our injury does not affect the hindlimb functions profoundly, the BBB rating does not adequately reflect the locomotor deficits in these animals. These results are consistent with another report that showed a rapid spontaneous recovery, as measured by both the BBB ratings and narrow-beam task after pyramidal tract lesion (19) but a more severe deficit in both tasks after a thoracic dorsal hemisection (20).

Time Course of Recovery

In our previous work using the cylinder test, we observed that the ability to use the affected forelimb either independently or together with the unaffected forelimb continued to improve through the first month after the transplantation (2). The cylinder primarily tests forelimb function and is therefore more vulnerable to a cervical lesion. Although hindlimb weight support is required to explore the cylinder wall, hindlimb digit control is not necessary. Moreover, in contrast to rope crossing, early after injury, the animal can preferentially use the unimpaired forelimb for vertical exploration without much adverse consequence. Vertical exploration is a normal part of the behavioral repertoire in the home cage. Self-regulated rehabilitation of the impaired forelimb for vertical exploration may be a gradual process because the nonimpaired forelimb can be used very effectively as a "crutch."

None of the tests used in the present study showed a significant improvement over the levels achieved at 2 weeks after surgery. The changes responsible for the different levels of function among the groups therefore must occur primarily within the first 2 weeks after surgery, suggesting a rapid reorganization of circuitry. This time course is compatible with sprouting, neural protection, and with the initiation of regeneration (21–23).

Repeated Lesion

The second lesion, even when placed close to the initial lesion, inevitably damages axons spared by the initial lesion. Nevertheless, after the second lesion, performance by the unmodified Fb or subtotal-hemisection groups was not significantly different from their performance after the first surgery on any task, indicating that the additional injury did not noticeably impair function. However, the Fb/BDNF group showed a marked decrease in their ability to perform on the rope-crossing, patch-removal, swim, and in our previous study, the cylinder tests after the second lesion. These results support a role for the transplant in promoting recovery.

The mechanisms underlying the transplant-mediated recovery of function, however, are not readily identified. Transplanted tissue, particularly when cells that produce neurotrophic factors are included, is likely to have a range of effects on the host tissue. In addition to promoting regeneration (reviewed in 24), and neuroprotection (reviewed in 25), sprouting of spared CNS pathways or of dorsal root axons can be elicited. We have shown growth of serotonergic and CGRP-labeled dorsal root axons as well as regeneration of rubrospinal axons into grafts of BDNF-secreting fibroblasts (2). Lateral funiculus lesions injure motor pathways; transplant-mediated growth of descending pathways can contribute to restoration of motor function, as in the cylinder and rope-crossing tests. Injury to the dorsal roots or to axons in Lissauer's tract may account for the sensory impairment seen in the patch-removal test. The sprouting or regeneration of cut dorsal root axons, particularly of the small-diameter axons that mediate crude touch, could account for restoration of sensory function (i.e., recognition of the patch) after the Fb/BDNF transplant.

We used a repeated-lesion strategy designed to eliminate the effect of axons that regenerate or sprout into the rostral portion of the transplant. The considerable reorganization of both cut and spared axonal systems, coupled with preservation of injured neurons and their continued contribution to spinal circuitry, may modify the intrinsic circuitry of the spinal cord in transplant recipients in unexpected ways. It is therefore difficult to predict the effect of a second lesion. A loss of function to a level comparable to that shown by operated controls, as in the cylinder test, will suggest that the recovered function requires the presence of these newly grown axons. Sprouting of intact axons or development of novel pathways by regenerating axons that are elicited by the neurotrophic factors from the graft might not be affected by the second lesion. If some of these behavioral modifications persist after a lesion that eliminates input to the graft, recovered performance dependent on the persisting changes may be little affected by the lesion, even though the anatomic changes are induced by and thus dependent on the presence of grafted tissue in the short term (5, 10). It also is possible that the spinal cord will become dependent on the modifications. In this case, a second lesion will have more profound effects on function. In the case of the rope and patch-removal tests, the performance of Fb/BDNF recipients after the second lesion was much worse than that of the hemisected or unmodified fibroblast recipients after either the first or second lesion.

In summary, the results suggest that the horizontal-rope test and the patch-removal tests might be particularly useful additions to a battery of tests used to evaluate the motor and sensory effects of intraspinal grafting of Fb/BDNF cells after subtotal hemisection of the cervical spinal cord, and that the enhanced outcome may reasonably be attributed to the presence of the transplant.

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