

Motor enrichment sustains hindlimb movement recovered after spinal cord injury and glial transplantation

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Abstract. *Purpose:* This study investigated whether enrichment improves hindlimb movement following complete spinal cord transection and transplantation of olfactory ensheathing glia (OEG), with or without a Schwann cell (SC) bridge.

Methods: Motor activity was encouraged through provision of motor enrichment housing (MEH); a multi-level cage containing ramps, textured surfaces and rewards. Hindlimb joint movement was assessed weekly for 22 weeks starting one week post-surgery, comparing rats housed in MEH to those in basic housing (BH). Transganglionic tracer was injected into the crushed right sciatic nerve three days prior to sacrifice, allowing sensory axons in the dorsal columns to be visualized by immunolabeling. Serotonergic axons and glial cells expressing low affinity nerve growth factor receptor were identified by immunolabeling.

Results: All rats, having received transplants, recovered some hindlimb movement. Rats housed in BH progressively lost recovered hindlimb function whereas recovered hindlimb movements were sustained in most rats in MEH. In rats transplanted with SCs and OEG, effects of MEH were first significant 14 weeks after injury. In rats transplanted with OEG, a trend was seen from 14 weeks after injury, but this did not reach significance. In all rats, traced sensory axons died back from sites of transplantation and did not regenerate rostrally. Further, in no rat were serotonergic axons observed regenerating into, around or beyond transplants.

Conclusions: Transection and transplantation of SC/OEG or OEG induced recovery of hindlimb function. This recovered hindlimb movement was sustained in rats housed in MEH but was progressively lost in rats housed in BH. Because benefits of MEH were not observed until 14 weeks after injury, long-term assessment of behavior is recommended. BH conditions are not conducive to maintenance of recovered hindlimb function, and MEH should be used in studies of recovery of function following spinal cord injury.

Keywords: Motor enrichment, rehabilitation, spinal cord transection, injury, hindlimb movement, Schwann cells, olfactory ensheathing glia

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1. Introduction

Successful treatment of human spinal cord injury (SCI) will likely require multiple interventions. In animal models of SCI, combinations of therapies often have proven more powerful than single treatments alone. Physical activity is attractive as a component of combination therapy for SCI repair. Evidence supporting the benefits of exercise and rehabilitation following SCI is accumulating [1–8].

Another promising therapeutic avenue for SCI repair is transplantation of cells including Schwann cells (SCs) and olfactory ensheathing glia (OEG). Following thoracic spinal cord transection in the adult rat, many CNS neurons extend axons into grafts of purified rat or human SCs [9–13]. Combined transplants of SCs and OEG further enhance axonal growth [14]. Furthermore, SCs can be obtained readily for autotransplantation and are neuroprotective after contusion injury [15].

OEG with or without SC bridges promote CNS axon regeneration and functional recovery after transplantation into the transected thoracic spinal cord [16–19]. In the study by Ramón-Cueto et al. [16], functional recovery was reported for a climbing task following intensive training and testing from three to seven months after surgery. This finding suggests that long-term rehabilitation may have improved recovery following transection and transplantation. Given the considerable evidence that rehabilitation improves outcome following SCI, we combined cell transplantation with motor activity to determine whether the combined effect would enhance functional recovery. Because many forms of motor therapy are extremely labor-intensive to conduct, we designed motor enrichment housing (MEH), an environment with opportunities for increased motor activity and weight bearing, in which rats participate according to individual ability and motivation. We hypothesized that rats in MEH would show enhanced functional recovery in comparison to rats housed individually in basic housing (BH) conditions.

2. Materials and methods

2.1. Animals

Experiments were performed using adult female Fischer 344 rats (Harlan Sprague Dawley, Indianapolis, Indiana) weighing 160–192 g, approximately 3 months of age. Prior to surgery, all rats were kept in pairs in standard plexiglas housing (described below) with food and water *ad libitum*. Rats were housed in a 12 h:12 h light:dark cycle. Approval was obtained from the University of Miami Animal Care and Use Committee.

2.2. Preparation of SC bridges

Schwann cells were purified (95–98%) from sciatic nerves according to methods described previously [20]. Briefly, segments of sciatic nerve from donor adult female Fischer rats were divested of perineurium and cultured on plastic to allow outgrowth of fibroblasts. Nerves were dissociated enzymatically and expanded using Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA) containing fetal calf serum (FCS, 10%), bovine pituitary extract (20 μ g/ml, Invitrogen), forskolin (0.8 μ g/ml, Sigma, St. Louis, MO) and heregulin (2.5 nM, Genentech, San Francisco, CA). Fibroblasts expressing Thy1.1 were also depleted by immunopanning. After re-plating after confluency three times, 1.83 million SCs were re-suspended in 8.37 μ l transplant medium (DMEM/F12 containing 0.1% gentamycin, Invitrogen) together with 8.33 μ l human plasma fibrinogen stock (100 mg/ml in transplant medium, Sigma), 0.42 μ l gentamycin stock (50 mg/ml, Sigma), 0.42 μ l calcium chloride stock (8% in distilled water) and 0.42 μ l bovine plasma aprotinin stock (78 IU per 62 μ l transplant medium, Sigma). Hollow polymer PAN/PVC (polyacrylonitrile/polyvinylchloride) channels (gift from Dr. Ty Hazlett) were cut into 3 mm lengths and 1 μ l of bovine plasma thrombin (25 units per μ l transplant medium, Sigma) was pipetted into each open end. The 18 μ l mixture was pipetted into the tubes prior to incubating at 37°C for ten minutes to allow gelling. Bridges were incubated in transplant medium overnight at 37°C with air containing 6% carbon dioxide. Bridges measuring 2 mm in length and 2.7 mm in diameter were dissected out of channels into Leibovitz-15 medium containing 0.1% gentamycin (L-15, Invitrogen) immediately prior to transplantation.

2.3. Preparation of OEG

Purified OEG were obtained from the nerve fiber layer of the adult rat olfactory bulb as described previously [15]. Briefly, olfactory bulbs were dissected from donor adult female Fischer rats, dissociated enzymatically and expanded as for SCs (except without using heregulin). p75 (nerve growth factor receptor) expressing cells were selected by immunopanning. Immediately prior to transplantation, aliquots of 400,000 OEG were suspended in 4 μ l of transplant medium (see above).

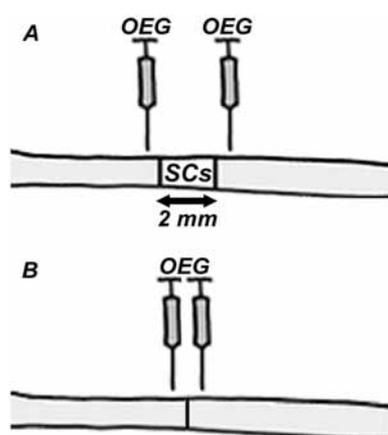


Fig. 1. Schematic depicting the two types of surgical intervention. Following thoracic transection of adult rat spinal cords, (A) a gap was created and a SC bridge was implanted with injection of OEG into the stumps; (B) OEG were injected into the stumps without accompanying SC bridge implantation.

2.4. Surgeries

Rats underwent one of two types of transection/transplantation surgery, shown schematically in Fig. 1. Rats were anesthetized by injection of 0.9% saline (0.06 ml per 100 g body weight, i.p.) containing ketamine hydrochloride (42.8 mg/ml, Phoenix Pharmaceuticals, St. Joseph, MO), xylazine hydrochloride (8.6 mg/ml, Phoenix) and acepromazine maleate (1.4 mg/ml, Phoenix). The surgical area was shaved and painted with 70% ethanol and betadine. Ophthalmic ointment (Lacrilube, Allergan Pharmaceuticals, Irvine, CA) was applied to the eyes to prevent drying. A midline incision was made along the thoracic vertebrae and skin and superficial muscles were retracted. Thoracic spinal cord vertebral laminae T8 to T10 were removed. The dura was incised at the dorsal midline, and lidocaine (1%, Abbott Laboratories, Abbott Park, IL, USA) was applied for 15 seconds.

In one group of rats, the T10 spinal cord was transected using angled microscissors. The cord was transected a second time caudally, and a segment was removed, creating a 2-mm gap centered in T10. A SC bridge, prepared as described above, was implanted into the gap to appose the stumps (Fig. 1A). Injections of OEG were made using sterile glass micropipettes attached to a 1- μ l syringe (Hamilton, Reno, NV) mounted on a stereotaxic micromanipulator. The spinal column was immobilized and four 0.5 μ l injections of OEG suspension were made into the cord midline, 1 mm from the end of each stump, at depths of 0.5, 1.0, 1.25 and 1.75 mm, to give a total of 200,000 cells per stump.

Two minutes were allowed to elapse to reduce reflux of cells on withdrawal of the micropipette. A small piece of gelfoam was left in place above the injection site.

In the second group of rats, the T10 spinal cord was transected. Care was taken to transect all ventral and lateral tissues, including the dura. Stumps were not lifted to confirm completeness of transection but in most rats they retracted slightly. OEG were injected into the cord stumps as described above (Fig. 1B).

A curved PAN/PVC roof, 4 mm in length, was placed on the site of transection and grafting to prevent adherence of the overlying tissues after suturing. Muscle layers were sutured closed, the skin was painted with antiseptic (3% hydrogen peroxide) and closed using Michel clips (Roboz, Gaithersburg, Maryland), which were removed 10 days after surgery. Saline (0.9%; 5 ml, s.c.) was given to prevent dehydration. For the first three days following surgeries, rats were given analgesics once daily (Tylenol, 1 ml containing 160 mg/kg body weight, p.o.). For the first 14 days post-surgery, rats were examined twice daily and given injections of prophylactic antibiotic (Cephazolin, 0.1 ml saline containing 30 mg/kg body weight, s.c.). Bladders were expressed manually twice daily until automatic voiding began. Rats were terminally anesthetized in the event of progressive, untreatable autophagia or autotomy, or excessive weight loss (> 20% of pre-operative body weight).

2.5. Post-operative housing

During the first seven days following surgery, rats were housed individually in plexiglas cages placed on heating pads (37°C) with water and food placed within cages for easy access. Absorbent bedding (Alpha-dri, Sherpherd, Watertown, TN) was placed on cage floors. Cages were changed twice weekly. Rats that survived one week post-surgery ($n = 38$) were randomly allocated to either basic housing (BH) or motor-enriched housing (MEH). There were no differences between BH and MEH groups in BBB score one week postinjury either for OEG (t-test, $p < 0.05$) or SC/OEG rats (t-test, $p < 0.05$) indicating that there were no differences in locomotor performance between groups at this time.

Figure 2(A) and Movie 1 depict BH, namely standard plexiglas cages measuring 17" long, 8" wide and 8" high. The cage roof allowed *ad libitum* access to food and water (via bottles with long, curved tips). Rats were housed individually. Figure 2(B) and Movie 2 depict MEH, a three-level ferret cage



Fig. 2. Rats were maintained postoperatively in one of two different types of housing. Digital photograph showing (A) basic and (B) motor enriched housing.

(Model 140, Midwest “Homes for Pets” distributed by www.theferretstore.com). The MEH set-up measured 36" long, 24" wide and 48" high and had a smooth plastic floor with two upper plastic shelves. Cage walls were made of 11-gauge wire (15/16 × 6 1/16"). This ferret cage, previously used successfully to improve function in rats with severe traumatic brain injury [21], was adapted for the present study in order to provide an environment where rats were encouraged to move freely, vigorously and without duress [avoiding potential adverse effects of early overuse of impaired limbs, which has been shown to be detrimental to recovery in several models of CNS injury [22–25]]. In addition, the MEH was designed to provide reinforcement for any movements that might occur due to recovering function, particularly hindlimb sweeping (extensive, alternating movements), retropulsion, partial weight support and plantar placement or stepping. Accordingly, inter-level ramps were set at angles of approximately 35 degrees above the horizontal. Plastic, non-toxic toys, tunnels and a running wheel (9" diameter, www.theferretstore.com) were placed on the ground floor. The running wheel was removed after two months because hindlimbs were seen to remain fully extended during use. A third upper level and an inter-level ramp (both consisting of wire grid) were

added after two months in an attempt to reinforce observed improvements in hindpaw movements. Placing bedding (crumpled paper towels) and treats (Froot Loops, Kellogg's) daily on the upper levels motivated rats to explore. Treat sticks (Hartz Mountain Corporation, Secaucus, NJ) were permanently hung from cage walls (6 to 12 inches above the first and second levels) to encourage climbing of wire walls. Food and water were placed at opposite sides of the ground floor, thereby encouraging movement but allowing *ad libitum* access. Rats were housed in groups of up to 12. MEH, therefore, provided not only environmental enrichment, cognitive challenge and social interactions, but also a specialized environment designed to encourage and reinforce any hindlimb movements. Photographs and movies were obtained in the nocturnal cycle when rats are most active.

Because rats in BH were kept in the same room as rats housed in MEH, all were exposed to the same light, humidity and temperature conditions. BH rats were handled regularly and given treats when the rats housed in MEH were given treats.

2.6. Behavioral testing

Locomotion was assessed using the BBB open-field test rating scale [26], by two observers blind to treat-

ment groups. BBB assessment was performed weekly for 22 weeks, beginning one week following transection/grafting. Only the 29 rats that survived until the end of the experiment were included for analysis.

2.7. Tract tracing

To label the central branch of sensory axons from the right hindlimb sciatic nerve, transganglionic tracer was injected after the last BBB test and three days before the end of the experiment. Rats were anesthetized using halothane (2%) in oxygen (0.6 liters/minute), the right hindlimb was shaved and painted with antiseptic, and the sciatic nerve was exposed at midhigh level. The nerve was crushed for 10 seconds using forceps, and 3 μ l of cholera toxin beta subunit solution (1% CTB, List Biologicals, Campbell, CA) were injected proximal to the crush. After three minutes, the needle (32-gauge) was withdrawn and the nerve was ligated distally.

2.8. Histology

At 22 weeks post-surgery, rats were terminally anesthetized and perfused transcardially, first with 100 ml phosphate buffered saline (PBS) and then with 500 ml 4% paraformaldehyde in PBS. Spinal cords were removed and stored overnight in fixative at 4°C prior to immersing for at least three days in cryoprotectant (30% sucrose in PBS) at 4°C.

Five series of twenty- μ m thick, horizontal 2 cm-long cryosections of spinal cord centered on the transection/transplant site were cut onto coated slides (Snow-coat X-tra, Surgipath, Richmond, IL) and were stored at -20°C. Immunolabeling was performed on slides using low-volume, low-evaporation chambers (Sequenza, Thermo Shandon, Pittsburgh, PA) according to manufacturer's instructions. Sections were thawed and air-dried fully prior to immunolabeling.

One series of sections was immunoperoxidase-labeled for CTB. Sections were rehydrated using buffer consisting of 100 mM Tris-HCl, 150 mM NaCl (TBS) and 2% Triton-X 100 (TXTBS). Endogenous peroxidase activity was quenched by incubating sections for 30 minutes in 3% hydrogen peroxide and 10% methanol. Sections were washed and blocked overnight using 2% TXTBS containing 5% heat-treated normal rabbit serum (NRS, Vector Laboratories, Burlingame, CA) prior to incubating for 60 hours in 2% TXTBS containing goat-anti CTB (1:20,000, List Biologicals) and 5% NRS. Sections were washed and then incubated for two hours in TBS containing bi-

otinylated rabbit anti-goat IgG (1:200, Vector) and 5% NRS. Sections were washed and then incubated for two hours in TBS containing streptavidin-horseradish peroxidase conjugate (Dako, Carpinteria, CA). Sections, washed twice in TBS and twice in 50 mM Tris-HCl (TNS), were immersed for 15 minutes in TNS containing 0.05% diaminobenzidine (Sigma) and 0.009% hydrogen peroxide. Sections were washed in TNS and immersed in cresyl violet staining solution (87 mM acetic acid containing 5 mM sodium acetate and 13 mg cresyl violet) for ten minutes prior to washing with distilled water, dehydrating through alcohols and coverslipping using Sub-X (Surgipath).

A second series of sections was immunofluorescence labeled for serotonin and p75. Sections were re-hydrated using 0.2% TXTBS and blocked overnight using 0.2% TXTBS containing 10% heat-inactivated normal goat serum (NGS). Sections were incubated overnight in 0.2% TXTBS containing rabbit polyclonal antibody against serotonin (1:500, Immunostar, Hudson, WI) and 1% NGS. Sections were washed and incubated for five hours in TBS containing goat anti-rabbit IgG conjugated to Alexa 594 (1:200, Molecular Probes, Eugene, OR) with 1% NGS. Sections were washed and blocked as before. Sections were incubated overnight in mouse monoclonal antibody against p75 (192 IgG supernatant from hybridoma cells supplied by Dr. Eugene Johnson, Washington University, St. Louis, MO) containing 5% NGS and 0.1% Tween-20. Sections were washed and then incubated for two hours in TBS containing goat anti-mouse IgG conjugated to Alexa 488 (1:200, Molecular Probes) and bisbenzimidazole. Sections were washed in PBS, air-dried and coverslipped using Vectamount (Vector Laboratories).

To confirm the presence of myelinated axons at the midpoint of SC bridges in a subset of SC/OEG rats ($n = 3$), a 1-mm thick transverse slice was taken for plastic embedding. To maximize use of available tissue, these specimens were obtained from rats that were terminally anesthetized or died from complications prior to the end of the experiment: these specimens were only used for identifying myelinated axons (and not for the other histology described above). For all other histology, cords were only obtained from rats that survived the full study and were perfusion fixed under terminal anaesthesia. For plastic embedding, cords were further fixed by immersion in glutaraldehyde and osmium tetroxide prior to dehydration and embedding in plastic. Semi-thin sections (1 μ m) were stained with toluidine blue [10]. The criteria to recognize peripheral-type myelin in plastic cross sections were the presence of a

signet ring configuration (myelin ring with the proximate SC nucleus) and the presence of space between the myelinated axons, which is caused by the formation of extracellular matrix. In contrast, central-type myelin profiles do not exhibit adjacent nuclei and have little or no space between them [15]. Sections were coverslipped using DEPEX (Electron Microscopy Sciences, Fort Washington, PA) and photographed (Axiovert, Zeiss) using a digital camera and PC software (NeuroLucida, MicroBrightfield, Williston, VT).

2.9. Statistical analysis

The surgery involved in the OEG groups (spinal cord transection) was different from that of the SC/OEG groups (removal of a two millimeter segment of spinal cord and implantation of a fibrin bridge containing SCs). Because the injury model is a factor in these experiments, differences between groups in mean BBB score were investigated using repeated measures analysis of variance (ANOVA) taking each injury type separately ($p = 0.05$). To identify the time at which differences between groups first became significant, the last ten time points were examined using *post hoc* *t*-tests with significance levels adjusted using Bonferroni's correction for multiple comparisons ($p < 0.005$). Variances were not assumed equal for groups because Levene's test for equality yielded $p < 0.05$ for most comparisons. Differences in the observed frequency of mortality between all four groups were examined using Pearson's Chi square test ($p = 0.05$). Differences in the observed frequency of mortality between paired subgroups or in crawling were examined using Fisher's exact test for 2×2 comparisons ($p = 0.05$). Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL).

3. Results

3.1. Mortality

Of the OEG-transplanted rats, 5 of 10 died in BH whereas 2 of 9 died in MEH. Of the SC/OEG-transplanted rats, 1 of 8 in BH died, whereas 1 of 11 in MEH died. These differences, however, were not significant (Pearson Chi square = 5.69, $df = 3$, asymmetric two sided $p = 0.128$). Of the nine rats that died after being transferred to BH or MEH, twice as many died when housed in BH ($n = 6$) as in MEH ($n = 3$). This difference, however, was not significant

(Fisher's exact test, one-tailed, $p = 0.17$). Of the rats that died, 7 had received OEG transplants and 2 had received SC/OEG transplants. This difference was not significant (Fisher's exact test, two-sided, $p = 0.124$).

3.2. Motor activity

Rats kept individually in BH were often sedentary throughout the 22-week survival period. In contrast, rats housed in MEH were highly active throughout the 22-week experiment. Digital movies illustrating these behaviours are available on request from L.M. Although communal housing of MEH rats prevented measurement of individual activity, the movie clearly shows that rats extensively used their forelimbs to climb ramps and the wire walls, as well as to climb over and through tunnels. Additionally, overnight eating of all treats provided on the upper cage levels served as an indirect measure of cage activity and indicated that rats were consistently motivated to climb to the second and third cage levels throughout the study. Hindpaws or tails did not become trapped in the rungs of the wheels or ramps. Hindlimbs were dragged behind the rats but were not entirely flaccid; joint movements were often seen in the hindlimbs of many animals, particularly when moving over textured ramps. Rats typically chose to sleep on the ground floor in groups of ten to twelve.

3.3. Hindlimb movement

Repeated measures ANOVA revealed an effect of housing for OEG-transplanted rats ($F_{1,10} = 5.57$; $p = 0.040$). Independent repeated measures ANOVA also revealed an effect of housing for SC/OEG-transplanted rats ($F_{1,15} = 1410$; $p < 0.001$). There was an effect of time for OEG-transplanted rats ($F_{21,210} = 31.1$; $p < 0.001$) and for SC/OEG-transplanted rats ($F_{21,315} = 35.7$; $p < 0.001$). There was also an interaction of housing with time for OEG-transplanted rats ($F_{21,210} = 2.51$; $p < 0.001$) and for SC/OEG-transplanted rats ($F_{21,315} = 5.61$; $p < 0.001$). There were, therefore, effects of both housing and time upon the score for open field movement in both OEG and SC/OEG-transplanted rats.

Inspection of BBB scores revealed that rats in all groups demonstrated some return of hindlimb joint movement after the spinal injury. Figure 3(A) depicts means and standard errors of the mean (SEM) of BBB scores for groups over time for all rats that survived 22 weeks post-surgery. Figure 3(B) depicts OEG transplanted groups and Fig. 3(C) depicts the SC/OEG trans-

Table 1
Percent of rats with a BBB score of at least seven

Group	1 week post-surgery	9 weeks post-surgery	14 weeks post-surgery	22 weeks post-surgery
OEG/BH	0	100	80	29
OEG/MEH	0	100	100	100
SC/OEG/BH	0	100	29	14
SC/OEG/MEH	0	100	100	60

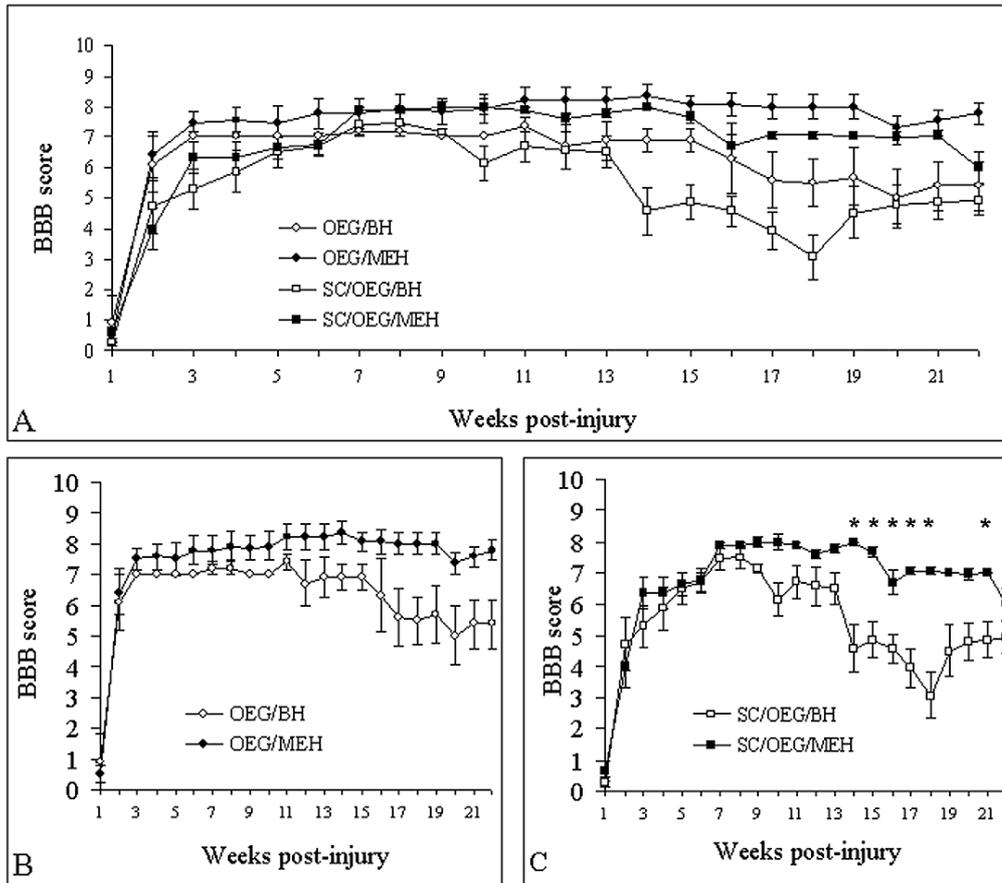


Fig. 3. Open field locomotor (BBB) testing showed that all groups of rats recovered a small degree of hindlimb function and that this was only maintained in groups of rats housed in motor enriched environments. Graphs showing means and standard errors of the mean (SEM) of averaged hindlimb BBB scores for (A) all rats that survived to 22 weeks postinjury, (B) all rats transplanted only with OEG, and (C) all rats transplanted with SCs and OEG. Asterisks indicate statistically significant differences between groups at a given time. Error bars are very small for the SC/OEG/MEH group as it had largest number of rats.

planted groups. One week post-surgery, all groups exhibited mean scores below one. However, by nine weeks, all rats had mean hindlimb scores of seven or more (Table 1), corresponding to extensive movement of all three joints of hindlimbs [26]. At 14 weeks post-surgery, 100% of rats housed in MEH still had BBB scores of at least seven whereas this was true only for 80% of OEG/BH and 29% of SC/OEG/BH rats. At 22 weeks post-surgery, 100% of OEG/MEH

rats and 60% of SC/OEG/MEH rats had scores equal to or greater than seven, whereas this was true for only 29% of OEG/BH rats and 14% of SC/OEG/BH rats (Table 1). Over the 22-week testing period, no BH rat ever attained mean hindlimb scores above eight. In contrast, three SC/OEG/MEH rats obtained peak mean hindlimb scores of nine and one OEG/MEH rat obtained 10.5. Indeed, this OEG/MEH rat consistently obtained a score of nine or more from three to 22 weeks

post-surgery. Several lines of evidence indicate that transection was complete in this rat. First, considerable care was taken during surgery to cut the spinal cord completely in all rats including dura and all lateral and ventral tissue. Second, one week post-surgery, this rat's mean hindlimb BBB score was 1.0. Third, histology revealed no evidence for sparing or regeneration across the injury site in this rat (see below).

To identify the time points at which differences between BH and MEH groups were significant, one-tailed t-tests were conducted from 13 to 22 weeks post-injury inclusive. These were conducted separately for SC/OEG-transplanted and OEG-transplanted rats (Tables 2 and 3, respectively).

When adjusted levels of significance were used ($p < 0.005$), differences were detected between BH and MEH in rats transplanted with SC/OEG at weeks 14–18 and 21 and trends were observed at weeks 19 and 20 ($p = 0.013$ and 0.0059 , respectively). At 14 weeks post-surgery, group scores (means \pm SEM) were 8.0 ± 0.0 for SC/OEG/MEH versus 4.6 ± 0.8 for SC/OEG/BH. At 22 weeks post-surgery, group mean scores were 6.0 ± 0.5 for SC/OEG/MEH versus 4.9 ± 0.5 for SC/OEG/BH. Thus SC/OEG-transplanted rats recover hindlimb locomotor function in both types of housing (BH and MEH). Rats in BH progressively lose recovered hindlimb function from 14 weeks post-surgery, whereas this is maintained for rats in MEH, at least until 21 weeks.

When adjusted levels of significance were used ($p < 0.005$), differences were not detected between BH and MEH rats transplanted with OEG at any timepoint from 13 to 22 weeks post-transplantation (Table 3). Inspection of mean differences and p values (Table 3) shows that hindlimb scores for MEH rats consistently exceeded those for BH but that this trend did not reach significance when levels of significance are adjusted using Bonferroni's correction. At 14 weeks post-surgery, group scores (means \pm SEM) were 8.4 ± 0.5 for OEG/MEH versus 6.9 ± 0.4 for OEG/BH. At 22 weeks post-surgery, group mean scores were 7.8 ± 0.3 for OEG/MEH versus 5.4 ± 0.8 for OEG/BH. In summary, OEG-transplanted rats recover hindlimb function in both types of housing (BH and MEH). OEG-transplanted rats in BH gradually lose function over time but a significant difference could not be detected at any specific timepoint.

During BBB testing, observers blind to treatment condition recorded the incidence of crawling, a hindlimb behavior that is not represented within the BBB rating scale. Crawling was defined as multiple

(> 3 cycles) alternating extensive movements of two or three hindlimb joints, with body weight being supported on the knee joints throughout. A digital movie illustrating this behaviour is available on request from L.M. Crawling was first noted in rats nine weeks after injury. Once established, crawling was stable, i.e., rats that exhibited crawling usually exhibited instances of crawling in all BBB testing sessions thereafter. Overall, 16 of 17 rats housed in MEH exhibited one or more instances of crawling relative to 5 of 12 rats housed in BH. Evaluation using a one-tailed Fisher's exact test revealed that this effect was significant ($p = 0.003$).

3.4. Growth of axons into SC bridges

To confirm that implantation of bridges containing SCs in fibrin had induced growth and myelination of axons, transverse semi-thin sections through SC grafts were studied ($n = 3$). Large numbers of axons grew into SC bridges and many bore peripheral myelin. Figure 4(A) depicts a region of a SC graft taken from a rat housed in MEH at 12 weeks after transplantation. Figure 4(B) shows peripherally myelinated axons at higher magnification. Grafts contained up to 13,279 SC-myelinated axons and up to 273 blood vessel profiles. Statistical comparisons between groups are not valid because only three cords were available for transverse semi-thin sectioning (cords from rats that had survived the full length of the study were entirely sectioned horizontally to examine whether axons grew longitudinally through grafts). These data are provided simply to confirm that SC transplants induced growth and myelination of axons, as seen in previous studies from this laboratory [13,27]. These axons are likely derived from sensory and spinal cord neurons with cell bodies proximal to transplants [10,13]. The functional significance of the presence of these axons within grafts has not yet been elucidated.

3.5. Sensory axons

To label the central branch of sensory axons from the right hindlimb sciatic nerve, a transganglionic tracer, CTB, was injected three days prior to the end of the experiment. Figure 5 shows that sensory axons ascending within the dorsal columns were completely interrupted by transection and, by 22 weeks post-surgery, had died back approximately 1 mm. Regeneration of these axons into transplantation sites was not seen in any rat, whether in SC/OEG or OEG groups, or whether housed in BH or MEH.

Table 2
Mean differences and p values for post hoc t-tests between BH and MEH groups for rats transplanted with SC/OEG

Week	Mean difference	Standard error	<i>t</i>	<i>df</i>	One tailed <i>p</i> value	Significant at <i>p</i> < 0.005?
13	-1.30	0.52	-2.51	6.86	0.020	No
14	-3.43	0.76	-4.52	6.00	0.002	Yes
15	-2.79	0.58	-4.79	7.33	0.001	Yes
16	-2.13	0.60	-3.55	12.66	0.002	Yes
17	-3.12	0.64	-4.87	6.41	0.001	Yes
18	-3.98	0.75	-5.29	6.30	0.001	Yes
19	-2.50	0.85	-2.96	6.00	0.013	No
20	-2.16	0.64	-3.36	7.14	0.006	No
21	-2.14	0.55	-3.87	6.00	0.004	Yes
22	-1.07	0.71	-1.51	14.59	0.076	No

Table 3
Mean differences and p values for post hoc t-tests between BH and MEH groups for rats transplanted with OEG

Week	Mean difference	Standard error	<i>t</i>	<i>df</i>	One tailed <i>p</i> value	Significant at <i>p</i> < 0.005?
13	-1.31	0.76	-1.73	7.10	0.063	No
14	-1.46	0.54	-2.72	9.07	0.012	No
15	-1.17	0.49	-2.41	7.58	0.022	No
16	-1.77	1.24	-1.43	4.86	0.107	No
17	-2.40	1.00	-2.40	5.34	0.029	No
18	-2.50	0.88	-2.85	5.83	0.015	No
19	-2.30	1.02	-2.26	5.30	0.035	No
20	-2.36	1.03	-2.30	5.11	0.034	No
21	-2.17	0.86	-2.51	5.43	0.025	No
22	-2.39	0.87	-2.75	5.49	0.018	No

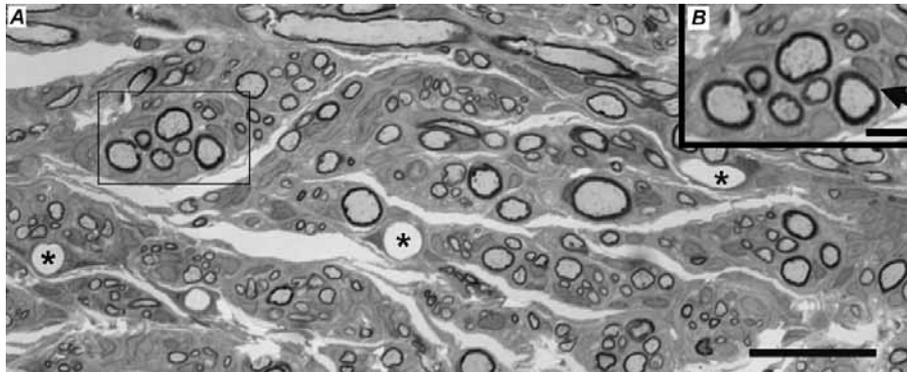


Fig. 4. Large numbers of peripherally myelinated axons were present in SC bridges. Micrograph showing a region within a semi-thin transverse section through a SC graft, taken from a SC/OEG rat that survived 12 weeks in MEH. Panel A illustrates peripherally myelinated axons throughout the graft. Blood vessels are also present (asterisks). Scale bar = 25 μ m. Panel B illustrates myelinated axons from the box in A shown at higher magnification; at the arrow, the SC nucleus is seen adjacent to the myelin, an identifying feature of SC-formed myelin. Scale bar = 5 μ m.

3.6. Serotonergic axons

Serotonergic axons were present in spinal cord rostral to sites of transection/transplantation but did not regenerate into or beyond injury sites in any rat, whether in SC/OEG or OEG groups or whether housed in BH or MEH. Figure 6(A) and (B), taken from an OEG/BH

rat, depicts serotonergic axons in spinal cord rostral to the site of transection, but not caudally. Serotonergic axons did not regenerate beyond the site of transection. Figure 6(C), taken from another OEG/BH rat, from an equivalent field of view, shows that serotonergic axons did not grow beyond the site of transection. Serotonergic axons did not grow amongst p75 immunoreac-

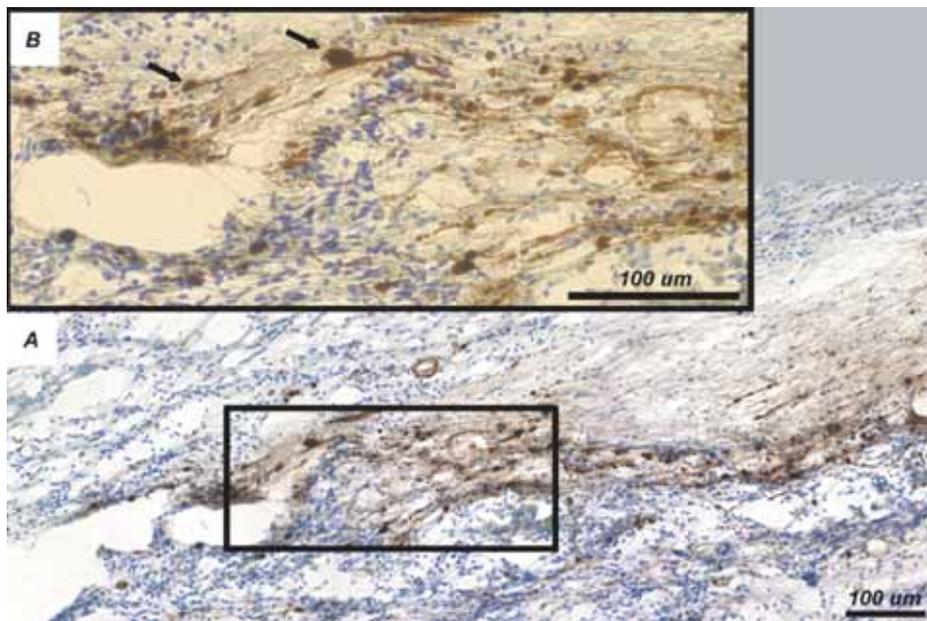


Fig. 5. Ascending central branches of sensory axons from lumbar DRG do not regenerate rostral to sites of complete transection and SC/OEG or OEG transplantation. Micrograph of a horizontal cryosection, immunoperoxidase-labeled for CTB (brown) and counterstained with cresyl violet (blue), taken from a SC/OEG/MEH rat at 22 weeks. The site of transection and SC implantation is 500 μm to the left side of the figure; the cord caudal to the SC bridge is shown. (A) Sensory axons die back 1 mm from the site of injury and have not regenerated rostrally. Scale bar = 100 μm . (B) Illustrates the boxed region in (A), showing retraction bulbs (arrows) at higher magnification. Scale bar = 100 μm .

tive cells (whether host SCs or transplanted OEG), despite their longitudinal orientation. Additionally, p75-immunoreactive cells were often present as chains of cells continuous with proximal spinal roots, suggesting that many SCs migrated into the injury site from the PNS (data not shown). In contrast to previous experiments [14], serotonergic axons were not observed regenerating in connective tissue surrounding SC bridges (data not shown).

4. Discussion

Rats were highly active in MEH, which afforded them many and varied opportunities for motor self-rehabilitation. In contrast, rats in BH were predominantly sedentary. MEH had a beneficial effect on hindlimb movements, assessed using BBB tests between one and 22 weeks post-surgery. Specifically, differences between BH and MEH rats were detected both for OEG-transplanted rats and for SC/OEG-transplanted rats.

Very modest improvements in hindlimb locomotor function were observed in all groups, whether maintained in BH or MEH. One week following surgery, all group mean BBB scores were below one, namely

flaccid paralysis with slight movement of one or two hindlimb joints. By nine weeks post-surgery every rat had a mean hindlimb score of at least seven, corresponding to extensive movement of all three joints of the hindlimb [26]. Over the 22-week testing period, no BH rat ever attained mean hindlimb scores above eight. In contrast, three SC/OEG/MEH rats obtained peak mean hindlimb scores of nine and one OEG/MEH rat obtained 10.5. Of special note, this OEG/MEH rat consistently obtained a score of nine or more from three to 22 weeks post-surgery. Thus, peak performance of BH rats corresponded to “Sweeping with no weight support, or plantar placement of the paw with no weight support” (BBB = 8). In contrast, peak performance of SC/OEG/MEH rats corresponded to “Plantar placement of the paw with weight support in stance only” or “Occasional, frequent or consistent weight supported dorsal stepping and no plantar stepping” (BBB = 9) while peak performance of OEG/MEH rats corresponded to “Occasional weight supported plantar steps with no forelimb-hindlimb coordination” (BBB = 10) [26].

Improvements in hindlimb movements were sustained up to 22 weeks in the majority of MEH rats. In contrast, hindlimb movements of the majority of rats in BH progressively deteriorated to scores below

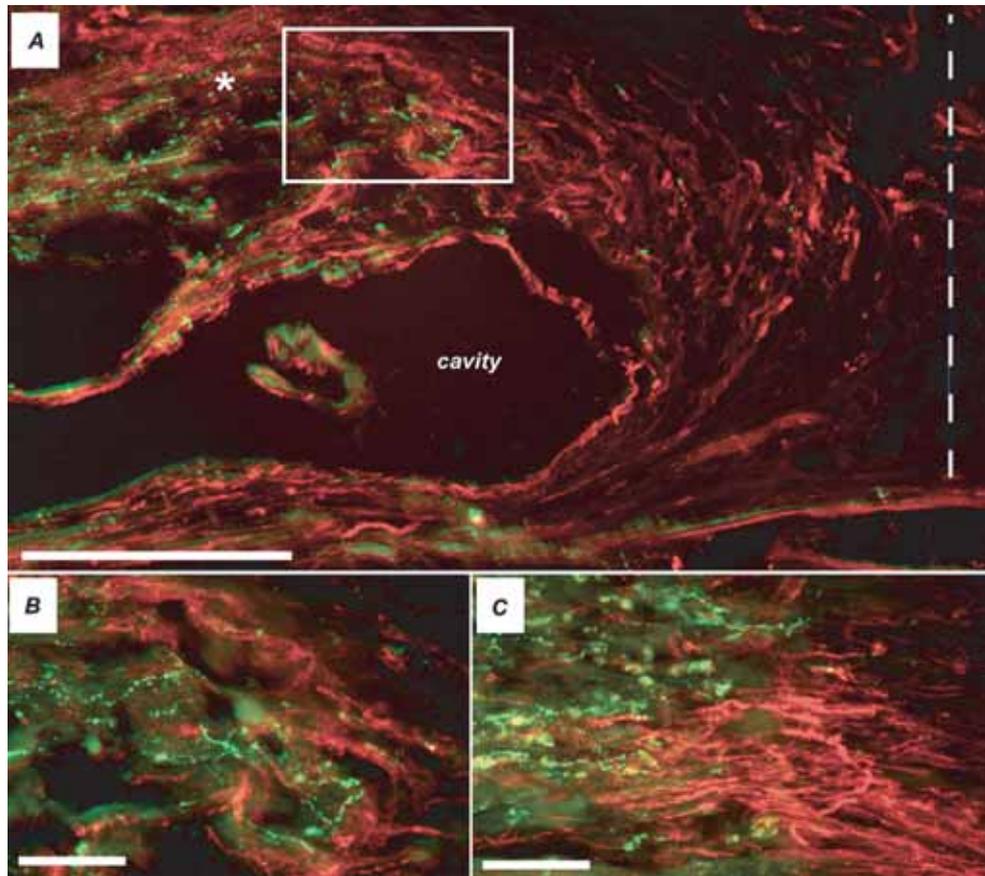


Fig. 6. Serotonergic axons do not regenerate caudal to sites of SC/OEG or OEG transplantation in either BH or MEH rats. Micrographs of horizontal cryosections immunolabeled for serotonin (green) and p75 nerve growth factor receptor (red), taken from OEG/BH rats, 22 weeks postsurgery. (A) Descending serotonergic axons were present in tissue rostral to sites of transection but not caudal to these sites. The dotted line indicates approximate level of transection. The asterisk indicates approximate level of injection of OEG. Scale bar = 500 μm . (B) Higher magnification of region boxed in (A). Serotonergic axons were only rarely associated with p75-immunoreactive cells. Scale bar = 100 μm . (C) Higher magnification of a similar region but from a different OEG/BH rat. Despite longitudinal arrangement of p75-immunoreactive cells, serotonergic neurons failed to extend into transplant sites. Scale bar = 100 μm .

7. Repeated measures ANOVA detected differences between BH and MEH rats both for OEG-transplanted and SC/OEG-transplanted rats. *Post hoc* tests of BBB data for 13 to 22 weeks post-surgery showed that the decline in function became significant 14 weeks post-surgery in SC/OEG/BH rats. The onset of decline was not detectable in OEG-transplanted rats using significance levels adjusted using Bonferroni's correction. The ability to detect a difference between BH and MEH rats transplanted with OEG by repeated ANOVA but not by numerous *post hoc* tests with significance levels adjusted for multiple testing may relate to differences in statistical power. In the SC/OEG comparison, there were 7 rats in BH and 10 rats in MEH. In the OEG comparisons, there were only 5 rats in BH and 7 in MEH. Smaller 'n's in the OEG groups result

in increased SEMs (standard deviation/square root of n) and a resulting loss of statistical power. Increased SEMs for the OEG groups relative to SC/OEG groups can be seen in Figures 3(B) and 3(C) respectively. The relatively small numbers of rats per group completing this study was an undesired consequence of maintaining rats with complete transection injuries (with transplants) for many months.

The decline in function detected in SC/OEG/BH rats may also relate to the more severe injury induced by removal of a two-millimeter segment of spinal cord in SC/OEG rats (relative to transection only in OEG rats). Degeneration in the spinal cord begins one millimetre more distally both rostrally and caudally after excision of a two millimetre segment: this may negatively influence lumbar spinal cord regions involved in hindlimb

function [28]. Because the rats in the present study were approximately one year old when the experiment was concluded, senescence was probably not a factor in causing the progressive decline.

Relative to rats housed in BH, more rats housed in MEH exhibited crawling. Crawling was defined as multiple (> 3 cycles), alternating, extensive movements of two or three hindlimb joints, with body weight being supported on the knees rather than paws. Crawling appeared reflexive, often being induced by sensory stimulation of the hindlimbs while rats used forelimbs to move over the various textured surfaces in the enriched environment, or by mechanical linkage of torso or forelimb movements. Transplantation may have induced crawling because historically we do not see this behaviour in non-transplanted rats with transected spinal cords.

The maximal mean BBB scores obtained by many of the OEG and SC/OEG rats in the present study are similar to or exceed those obtained in some other studies using thoracic complete transection as an injury model. These include 1) transplantation of glia obtained from olfactory mucosa (mean BBB not stated but approximately 5.5 at eight weeks after implantation [17]), 2) activated monocytes (mean BBB for *posthoc* subgroup at 19 weeks = 7.1 [29]), 3) implantation into the transection gap of peripheral nerve grafts secured with fibrin glue containing acidic fibroblast growth factor (mean BBB at six months = 6.8 [30] and 7.1 [31]; 4) implantation of SC bridges and OEG with delivery of IgG and chondroitinase ABC (BBB at nine weeks = 6.6 [19]), or 5) implantation of SCs into nude rats treated with steroids (maximal mean BBB reported at 6 weeks = 8.2 [32]).

In several of these studies, one or more transplanted rats achieved scores of 10 yet after thoracic transection and intervention of any kind, plantar placement, weight support of the hindlimbs and/or forelimb-hindlimb coordination remains the exception. To our knowledge, only one study reports high incidences of plantar placement and weight-supported stepping in rats with transection injuries (although conventional BBB testing was not conducted). This study combined grafting of fetal tissue two weeks post-injury with neurotrophin treatment [33]. In conclusion, more work remains to be done to obtain consistent plantar placement and weight-supported stepping in adult rats with complete transection injury.

Transection-only controls were not included in the present study because experience in our laboratory and elsewhere has shown that adult rats with complete

thoracic spinal cord transections rarely exhibit extensive movements of more than a single hindlimb joint (BBB score < 2) [17–19,26,29], even up to one year post-injury [34]. Improvements in open field hindlimb movement are not seen in transected non-transplanted rats even following long-term training/testing on an inclined plane task [16]. Motor enrichment therapies provide only slight benefit in open field locomotion in much milder forms of injury, such as following moderate thoracic contusion [1]. We thus regarded it unlikely that enrichment would provide significant improvement to transected rats without grafts. We emphasize that our conclusions in this study are based on comparisons between BH and MEH housed rats transplanted either with OEG or SCs with OEG, without the need for comparison to transection-only controls.

Recovery of hindlimb movements after transection has been linked to regrowth of serotonergic fibers to lumbar spinal regions [16,35]. In the present study, we observed recovery of hindlimb function comparable to that reported in these studies (up to a BBB of 10.5) but without evidence of regeneration of descending serotonergic axons. Although our method of OEG preparation may differ from that of other groups, serotonergic regeneration after transection has been reported using OEG prepared in our laboratory [19]. In the present study, however, transections were slightly more caudal than in previous studies and, whereas in other transection/OEG transplantation studies ventral dura was spared [17], ventral dura was cut in our transection/OEG rats. The present study also grafted SCs within fibrin rather than Matrigel [14,19] and implanted a polymer hemichannel roof above grafts rather than an intact polymer channel that enclosed grafts. Serotonergic fibers were not seen in connective tissue on the roof's external surface in contrast to the presence of these fibers around intact channels [14]; in the latter study, fibers re-entered the spinal cord caudal to the channel/SC transplant. We also found no evidence for regeneration of ascending central branches of sensory axons from L3-L6 DRG. This result is consistent with previous work from this laboratory [13], which showed that very few sensory axons whose cell bodies lie in L3-L6 DRG (as would be traced by the CTB injection) extend axons into SC bridges placed at T8-T10 one month previously.

Given the lack of evidence for regrowth of serotonergic fibers or dorsal column sensory axons, other mechanisms must explain the recovery of hindlimb movements in BH and MEH rats. It is possible that fiber tracts other than those examined in the present

study (e.g. reticulospinal axons) regenerated across the site of transection and reinnervated distal cord. It is entirely possible, however, that improvements in hindlimb movement can proceed without axon regeneration across the injury site but instead from changes in circuitry below the level of transection. This might include compensatory plasticity of networks in the lumbar spinal cord such as modulation of existing synaptic connections. For example, after transection in rats and mice, pharmacological or electrophysiological stimulation can induce partial weight-supported stepping on treadmills in the absence of regeneration of descending or ascending fiber tracts [28,36]. Transplanted SCs or OEG are neuroprotective [15] and factors secreted by grafts might influence function of spared circuits in the spinal cord caudal to the transection such as the central pattern generator. We hypothesize that function recovered in BH and MEH rats was sustained in MEH rats due to regenerated axons and/or reorganized circuitries being maintained/reinforced through sensory stimulation during motor activity.

Our study shows that housing rats in stimulating environments confers a number of benefits. The loss of restored function in rats in long-term (> two months) BH implies that “standard” plexiglas cages should instead be viewed as “sub-standard” or “impoverished”, whereas “enriched environments” should arguably replace “basic housing”. It has been known for decades that depriving rats of social contact with other rats leads to physiological and behavioral abnormalities [39]. More recent studies have shown that impoverished environments hinder recovery after brain injury [40,41] and change gait characteristics in intact rats [42]. Our study shows that a small degree of hindlimb function was recovered in rats housed in BH comparable to that recovered by rats in MEH, but that BH housing led to reduced BBB scores overall. Our study thus provides yet more evidence that single housing of rats in cages devoid of enrichment opportunities must be avoided in the laboratory setting, particularly for studies concerning functional recovery after injury to the CNS. It is also important to note that the progressive detrimental effect upon restored function began more than two months post-surgery, emphasizing the need to monitor recovery for more than just one or two months.

To our knowledge, this study is the first to combine glial cell transplantation following complete transection injury with motor enrichment. Other studies have demonstrated beneficial effects of exposure to targeted motor therapy and environmental enrichment in other models of brain or spinal cord injury [1–6,43–46].

Our results further support the notion that recovery of function is enhanced by locomotor activity. Furthermore, although it has been shown that environmental enrichment can delay symptom onset of neurodegenerative disease [47], as well as reverse aging-associated neurodegenerative events [48], the present work is the first study that we know to indicate that post-operative enrichment can prevent progressive loss of *restored* hindlimb movement.

In sum, our study demonstrates that long-term maintenance in deprived circumstances can lead to progressive loss of residual or restored function, whereas opportunities for self-rehabilitation and social interaction can sustain recovered function following spinal transection and acute glial cell transplantation. More work needs to be done to understand the conditions under which motor rehabilitation and/or transplantation of OEG and SCs will provide significant benefit in humans. In the interim, our results suggest that spinal cord injured persons should be encouraged to explore rehabilitation opportunities for improving overall health and maintaining or improving residual function.

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References

- [1] A.J. Lankhorst, M.P. ter Laak, T.J. van Laar, N.L. van Meeteren, J.C. de Groot, L.H. Schrama et al., Effects of enriched housing on functional recovery after spinal cord contusion injury in the adult rat, *Journal of Neurotrauma* **18** (2001), 203–215.
- [2] J.W. McDonald, D. Becker, C.L. Sadowsky, J.A. Jane, Sr., T.E. Conturo and L.M. Schultz, Late recovery following spinal cord injury. Case report and review of the literature, *Journal of Neurosurgery* **97** (2002), 252–265.
- [3] R.D. de Leon, J.A. Hodgson, R.R. Roy and V.R. Edgerton, Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats, *Journal of Neurophysiology* **79** (1998), 1329–1340.

- [4] A.L. Behrman and S.J. Harkema, Locomotor training after human spinal cord injury: a series of case studies, *Physical Therapy* **80** (2000), 688–700.
- [5] E.C. Field-Fote and D. Tepavac, Improved intralimb coordination in people with incomplete spinal cord injury following training with body weight support and electrical stimulation, *Physical Therapy* **82** (2002), 707–715.
- [6] E. Taub, G. Uswatte and R. Pidikiti, Constraint-induced movement therapy: a new family of techniques with broad application to physical rehabilitation – a clinical review, *Journal of Rehabilitation Research and Development* **36** (1999), 237–251.
- [7] K.J. Hutchinson, F. Gomez-Pinilla, M.J. Crowe, Z. Ying and D.M. Basso, Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats, *Brain* **127** (2004), 1403–1414.
- [8] A. Wernig and S. Muller, Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries, *Paraplegia* **30** (1992), 229–238.
- [9] X.M. Xu, V. Guenard, N. Kleitman, P. Aebischer and M.B. Bunge, A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord, *Experimental Neurology* **134** (1995), 261–272.
- [10] X.M. Xu, V. Guenard, N. Kleitman and M.B. Bunge, Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord, *Journal of Comparative Neurology* **351** (1995), 145–160.
- [11] J.D. Guest, D. Hesse, L. Schnell, M.E. Schwab, M.B. Bunge and R.P. Bunge, Influence of IN-1 antibody and acidic FGF-fibrin glue on the response of injured corticospinal tract axons to human Schwann cell grafts, *Journal of Neuroscience Research* **50** (1997), 888–905.
- [12] M. Oudega, X.M. Xu, V. Guenard, N. Kleitman and M.B. Bunge, A combination of insulin-like growth factor-I and platelet-derived growth factor enhances myelination but diminishes axonal regeneration into Schwann cell grafts in the adult rat spinal cord, *Glia* **19** (1997), 247–258.
- [13] X.M. Xu, A. Chen, V. Guenard, N. Kleitman and M.B. Bunge, Bridging Schwann cell transplants promote axonal regeneration from both the rostral and caudal stumps of transected adult rat spinal cord, *Journal of Neurocytology* **26** (1997), 1–16.
- [14] A. Ramon-Cueto, G.W. Plant, J. Avila and M.B. Bunge, Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants, *Journal of Neuroscience* **18** (1998), 3803–3815.
- [15] T. Takami, M. Oudega, M.L. Bates, P.M. Wood, N. Kleitman and M.B. Bunge, Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord, *Journal of Neuroscience* **22** (2002), 6670–6681.
- [16] A. Ramon-Cueto, M.I. Cordero, F.F. Santos-Benito and J. Avila, Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia., *Neuron* **25** (2000), 425–435.
- [17] J. Lu, F. Feron, S.M. Ho, A. Mackay-Sim and P.M. Waite, Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats, *Brain Research* **889** (2001), 344–357.
- [18] J. Lu, F. Feron, A. Mackay-Sim and P.M. Waite, Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord, *Brain* **125** (2002), 14–21.
- [19] K. Fouad, L. Schnell, M.B. Bunge, M.E. Schwab, T. Liebscher and D.D. Pearce, Combining Schwann cell bridges and olfactory ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord, *Journal of Neuroscience* **25** (2005), 1169–1178.
- [20] T.K. Morrissey, N. Kleitman and R.P. Bunge, Isolation and functional characteristics of Schwann cells derived from adult peripheral nerve, *Journal of Neuroscience* **11** (1991), 2433–2442.
- [21] M.J. Passineau, E.J. Green and W.D. Dietrich, Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats, *Experimental Neurology* **168** (2001), 373–384.
- [22] J.L. Humm, D.A. Kozlowski, S.T. Bland, D.C. James and T. Schallert, Use-dependent exaggeration of brain injury: is glutamate involved? *Experimental Neurology* **157** (1999), 349–358.
- [23] S. Stoltz, J.L. Humm and T. Schallert, Cortical injury impairs contralateral forelimb immobility during swimming: a simple test for loss of inhibitory motor control, *Behavioral Brain Research* **106** (1999), 127–132.
- [24] D.A. Kozlowski, D.C. James and T. Schallert, Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions, *J Neuroscience* **16** (1996), 4776–4786.
- [25] S.T. Bland, T. Schallert, R. Strong, J. Aronowski, J.C. Grotta and D.M. Feeney, Early exclusive use of the affected forelimb after moderate transient focal ischemia in rats: functional and anatomic outcome, *Stroke* **31** (2000), 1144–1152.
- [26] D.M. Basso, M.S. Beattie and J.C. Bresnahan, A sensitive and reliable locomotor rating scale for open field testing in rats, *Journal of Neurotrauma* **12** (1995), 1–21.
- [27] M.F. Meijs, L. Timmers, D.D. Pearce, P.A. Tresco, M.L. Bates, E.A. Joosten et al., Basic fibroblast growth factor promotes neuronal survival but not behavioral recovery in the transected and Schwann cell implanted rat thoracic spinal cord, *J Neurotrauma* **21** (2004), 1415–1430.
- [28] R.M. Ichiyama, Y.P. Gerasimenko, H. Zhong, R.R. Roy and V.R. Edgerton, Hindlimb stepping movements in complete spinal rats induced by epidural spinal cord stimulation, *Neuroscience Letters* **383** (2005), 339–344.
- [29] O. Rapalino, O. Lazarov-Spiegler, E. Agranov, G.J. Velan, E. Yoles, M. Fraidakis et al., Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats, *Nature Medicine* **4** (1998), 814–821.
- [30] Y.S. Lee, I. Hsiao and V.W. Lin, Peripheral nerve grafts and aFGF restore partial hindlimb function in adult paraplegic rats, *Journal of Neurotrauma* **19** (2002), 1203–1216.
- [31] Y.S. Lee, C.Y. Lin, R.T. Robertson, I. Hsiao and V.W. Lin, Motor recovery and anatomical evidence of axonal regrowth in spinal cord-repaired adult rats, *Journal of Neuropathology and Experimental Neurology* **63** (2004), 233–245.
- [32] J.D. Guest, A. Rao, L. Olson, M.B. Bunge and R.P. Bunge, The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord, *Experimental Neurology* **148** (1997), 502–522.
- [33] J.V. Coumans, T.T. Lin, H.N. Dai, L. MacArthur, M. McAttee, C. Nash et al., Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins, *Journal of Neuroscience* **21** (2001), 9334–9344.
- [34] H. Cheng, Y. Cao and L. Olson, Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function, *Science* **273** (1996), 510–513.

- [35] M. Gimenez y Ribotta, J. Provencher, D. Feraboli-Lohnherr, S. Rossignol, A. Privat and D. Orsal, Activation of locomotion in adult chronic spinal rats is achieved by transplantation of embryonic raphe cells reinnervating a precise lumbar level, *Journal of Neuroscience* **20** (2000), 5144–5152.
- [36] A.J. Fong, L.L. Cai, C.K. Otsoshi, D.J. Reinkensmeyer, J.W. Burdick, R.R. Roy et al., Spinal cord-transected mice learn to step in response to quipazine treatment and robotic training, *Journal of Neuroscience* **25** (2005), 11738–11747.
- [37] C.E. Hill, L.D. Moon, P.M. Wood and M.B. Bunge, Labeled Schwann cell transplantation: Cell loss, host Schwann cell replacement, and strategies to enhance survival, *Glia* **53** (2006), 338–343.
- [38] D.J. Barakat, S.M. Gaglani, S.R. Neravetla, A.R. Sanchez, C.M. Andrade, Y. Pressman et al., Survival, integration, and axon growth support of glia transplanted into the chronically contused spinal cord, *Cell Transplantation* **14** (2005), 225–240.
- [39] G.S. Wiberger and H.C. Grice, Long-term isolation stress in rats, *Science* **142** (1963), 507.
- [40] B. Will, F. Deluzarche and C. Kelche, Does post-operative environment attenuate or exacerbate symptoms which follow hippocampal lesions in rats? *Behavioural Brain Research* **7** (1983), 125–132.
- [41] B. Will, G. Toniolo, C. Kelche, V. Pallage, F. Deluzarche and R. Misslin, The effects of postoperative physical environment on novelty seeking behaviour and maze learning in rats with hippocampal lesions, *Behavioural Brain Research* **19** (1986), 233–240.
- [42] L. Roberts, K.A. Clarke and J.R. Greene, Post-weaning social isolation of rats leads to an abnormal gait, *European Journal of Neuroscience* **13** (2001), 2009–2012.
- [43] A. Thota, S. Carlson and R. Jung, Recovery of locomotor function after treadmill training of incomplete spinal cord injured rats, *Biomedical Scientific Instruments* **37** (2001), 63–67.
- [44] K. Fouad, G.A. Metz, D. Merkler, V. Dietz and M.E. Schwab, Treadmill training in incomplete spinal cord injured rats, *Behavioural Brain Research* **115** (2000), 107–113.
- [45] R.J. Nudo, B.M. Wise, F. SiFuentes and G.W. Milliken, Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct, *Science* **272** (1996), 1791–1794.
- [46] J.A. Kleim, T.A. Jones and T. Schallert, Motor enrichment and the induction of plasticity before or after brain injury, *Neurochemistry Research* **28** (2003), 1757–1769.
- [47] T.L. Spires, H.E. Grote, N.K. Varshney, P.M. Cordery, A. van Dellen, C. Blakemore et al., Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism, *Journal of Neuroscience* **24** (2004), 2270–2276.
- [48] H. Hilbig, H.J. Bidmon, S. Steingruber, H. Reinke and H.R. Dinse, Enriched environmental conditions reverse age-dependent gliosis and losses of neurofilaments and extracellular matrix components but do not alter lipofuscin accumulation in the hindlimb area of the aging rat brain, *Journal of Chemical Neuroanatomy* **23** (2002), 199–209.