Published in final edited form as:
Exp Neurol. 2010 August ; 224(2): 429–437. doi:10.1016/j.expneurol.2010.05.003.

Improvement of gait patterns in step-trained, complete spinal cord-transected rats treated with a peripheral nerve graft and acidic fibroblast growth factor

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Abstract

The effects of peripheral nerve grafts (PNG) and acidic fibroblast growth factor (αFGF) combined with step training on the locomotor performance of complete spinal cord-transected (ST, T8) adult rats were studied. Rats were assigned randomly to five groups (N = 10 per group): sham control (laminectomy only), ST only, ST–step-trained, repaired (ST with PNG and αFGF treatment), or repaired–step-trained. Step-trained rats were stepped bipedally on a treadmill 20 min/day, 5 days/week for 6 months. Bipolar intramuscular EMG electrodes were implanted in the soleus and tibialis anterior (TA) muscles of ST–step-trained (n = 3) and repaired–step-trained (n = 2) rats. Gait analysis was conducted at 3 and 6 months after surgery. Stepping analysis was completed on the best continuous 10-s period of stepping performed in a 2-min trial. Significantly better stepping (number of steps, stance duration, swing duration, maximum step length, and maximum step height) was observed in the repaired and repaired–step-trained than in the ST and ST–step-trained rats. Mean EMG amplitudes in both the soleus and TA were significantly higher and the patterns of activation of flexors and extensors more reciprocal in the repaired–step-trained than ST–step-trained rats. 5-HT fibers were present in the lumbar area of repaired but not ST rats. Thus, PNG plus αFGF treatment resulted in a clear improvement in locomotor performance with or without step training. Furthermore, the number of 5-HT fibers observed below the lesion was related directly to stepping performance. These observations indicate that the improved stepping performance in Repaired rats may be due to newly formed supraspinal control via regeneration.

Keywords

Spinal cord injury; Nerve regenereation; Step training; Skeletal muscle activity

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Introduction

One important approach used in attempts to regain functional recovery after a spinal cord injury (SCI) has included transplantation strategies that are designed to provide a favorable environment for regrowth, e.g., Schwann cell, olfactory ensheathing cell, or peripheral nerve segment implants at or near the lesion site (Xu et al., 1995; Ramon-Cueto et al., 2000; Cheng et al., 1996; Kubasak et al., 2008). Other strategies including the application of growth factors or the removal of inhibitory molecules also may stimulate some axonal regrowth in SCI animals (for review, Silver and Miller, 2004; Sharma, 2007).

A favorable environment for axon regeneration after a complete spinal transection can be created by a combination of a peripheral nerve graft (PNG) transplantation and acidic fibroblast growth factor (αFGF) treatment (Cheng et al., 1996; Tsai et al., 2005). We have reported that this combinatory treatment in adult complete spinal rats results in (1) significant improvement in BBB open field test scores (Lee et al., 2002b), (2) partial (30% of normal) recovery of motor-evoked potentials (Lee et al., 2002b), (3) regrowth of corticospinal tract fibers (Lee et al., 2004a), (4) the presence of serotonergic fibers beyond the lesion site (Lee et al., 2004a), and (5) a reduction in the loss of soleus muscle mass and the maintenance of its slow phenotype near normal (Lee et al., 2007).

In addition to the stimulation of the regrowth of damaged fibers, rehabilitative therapies alone or combined with other interventions have been used to promote locomotor performance in SCI animals (Edgerton et al., 2006). Weight-supported treadmill training was initially tested in cats and was found to significantly enhance hindlimb stepping ability after a complete midthoracic spinal cord transection, indicating that the lumbar spinal cord had learned to step (Lovely et al., 1990; Edgerton et al., 1997; de Leon et al., 1998, 1999). Furthermore, adult spinal rats show improved stepping performance on the treadmill when trained to step (Moshonkina et al., 2002; Timoszyk et al., 2003). Several lines of evidence indicate that the modulation of sensory input from the legs during training plays a significant role in the reorganization of the spinal circuits that generate stepping (Edgerton et al., 1997; Cha et al., 2007).

The purpose of the present study was to examine the effects of PNG and αFGF treatment with a more rigorous assessment of the stepping ability (kinematics and EMG) and the potential role of regrowth of serotonergic fibers after a complete, midthoracic spinal cord transection in adult rats. We also tested whether the effect of combination of PNG and αFGF plus step training would have an additive effect on motor behavior or on the regeneration of serotonergic fibers.

Materials and methods

Animal groups

Fifty adult female Sprague-Dawley rats (225-250 g; Harlan, San Diego) were assigned randomly and equally into five groups (10 rats/group): (1) sham (laminectomy only), (2) ST (T8 spinal cord transection only), (3) ST–step trained, (4) repaired (ST with PNG and αFGF treatment), and (5) repaired–step-trained. Three rats in the ST group, one rat in the ST–step-trained group, and three rats in the repaired group died prematurely. Therefore, a total of 43 rats completed the entire sequence of tests, i.e., 10, 7, 9, 7, and 10 rats in the five groups, respectively. All animal procedures followed the NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the Veteran Affairs Long Beach Healthcare System. Animals were housed in ventilated, humidity-controlled (50%), and temperature-controlled (23–25 °C) rooms on a 12:12-hour light–dark cycle.

Spinal cord surgery and peripheral nerve transplantation

All surgical procedures were performed under aseptic conditions. Before surgery, all rats were anesthetized using sodium pentobarbital (40 mg/kg, i.p.). The rats were maintained on a heating pad and the rectal temperature was monitored and maintained within ± 1.5 °C of normal temperature during surgery. Bipolar electrocauterization was used to minimize bleeding. Rats in the Sham group solely underwent a partial laminectomy at the T8 level. Rats in the ST and ST–step-trained groups underwent a similar partial laminectomy followed by two complete transverse cuts of the spinal cord at approximately the T8 level, creating a gap of ~5 mm. A surgical microscope was used to ensure the complete removal of neural tissue from the 5 mm gap (nothing was inserted in this gap). Rats in the repaired and repaired–step-trained groups underwent a spinal cord transection as described above, except that the 5-mm gap was repaired using PNG–autotransplantation and αFGF treatment as described previously (Lee et al., 2002b; Lee et al., 2004b). Briefly, 18 intercostal nerve segments were harvested and transplanted into the 5-mm gap to bridge the white matter (proximal site) to the gray matter (distal site). A mixture of αFGF (1 μg; R&D Systems, Minneapolis, MN, USA) and fibrin glue was applied on top of the grafts. The vertebral column was fixed in a dorsiflexed position using a compressive S-shaped monofilament surgical steel wire (B&S gauge 20; DS-20, Ethicon) loop fastened to the vertebral column with nonabsorbable threads. The muscle layers were closed using 4-0 plain nature gut suture. The skin was closed using 2-0 sutures. Buprenorphine administration (0.05 mg/kg, s.c. twice a day), beginning immediately before surgery and continuing for 2 days after operation, was used for analgesia. The bladders of all spinal cord-transected rats were expressed manually at least twice per day throughout the experimental period. Heating pads were placed beneath the plastic cages during the first 3 days after surgery.

Step training and gait analyses

Step-trained rats received manual bipedal step training on the treadmill (20 min/day, 5 days/week for 6 months) beginning 3 weeks after spinal cord surgery. A robotic-assisted device (Robomedica Inc., Irvine, CA) was used to train the hindlimbs to step as described previously (de Leon and Acosta, 2006). The device includes (1) two lightweight robotic arms attached to the ankles, (2) a body weight-support apparatus that is used to secure the body weight support arm through cloth vest/Velcro fasteners and to control the amount of load on both hindlimbs of the rat, and (3) a motorized treadmill. Gait analysis was conducted at 3 and 6 months after surgery using the robot-assisted device to track the two-dimensional movements of the ankle during stepping. Analysis was performed on the best continuous 10-s period of stepping on the treadmill at 13.5 cm/s. The following kinematics data were collected: (1) total number of steps, (2) mean cycle period duration, (3) mean stance duration, (4) mean swing duration, (5) mean step length, (6) maximum step length, ((7) mean step height, (8) maximum step height, (9) principal component analysis (PCA) of the hindlimb movement when moving in the horizontal direction (XPCA), (10) PCA in the vertical direction (YPCA), and (11) full-width at half-maximum value of the Fast Fourier Transform (FTT) of the hindlimb movement when moving in the horizontal direction.

EMG recordings from the soleus and tibialis anterior muscles in ST–step-trained and repaired–step-trained rats

After the completion of all gait analyses, the soleus and tibialis anterior (TA) muscles of three rats in the ST–step-trained and two rats in the repaired–step-trained groups were implanted with bipolar intramuscular chronic EMG electrodes as described previously (Roy et al., 1991). Briefly, a head connector was secured to the skull using screws and dental cement and two Teflon-coated wires were passed subcutaneously from the head connector to each of the muscle sites bilaterally. The wires were inserted into the muscle belly using a 23-gauge needle, and a small notch (~0.5–1.0 mm) of Teflon was removed from each wire to

form the recording electrodes. Once proper placement of the electrodes was verified by back-stimulation through the head connector, the wires were secured at their entrance into and exit from the muscle with sutures. Two weeks after the EMG implants, the rats were tested for locomotor ability. The same body weight support system and treadmill were used and the movements of the limbs were detected using 3D motion kinematics (SIMI Motion, zFlo, Lexington, MA). Retroreflective markers were placed at the iliac crest, hip, knee, ankle, and metatarsophalangeal joints bilaterally. EMG data were passed through a differential amplifier (Model 1700; A-M Systems, Sequim, WA) adjusted to a gain of ×1000 and acquired on a standard laboratory computer at 2 kHz. Analysis was performed on the best continuous 10-s period of stepping on the treadmill at 13.5 cm/s.

Immunohistochemical identification and quantification of 5-HT fibers in the spinal cords

After perfusion, the spinal cords from all rats were immersed in a 20% sucrose solution. A microtome was used to section the spinal cords at a 40-μm thickness in both the horizontal and transverse planes. The horizontal plane sections were collected from the graft–spinal cord area to examine the distribution of 5-HT fibers at the interface of the PNG and the distal end of the spinal cord tissue. The transverse plane sections were collected from the lumbar area to determine the presence of 5-HT fibers. For quantification of 5-HT immunoreactivity, the number of labeled axons was counted on sections from both the horizontal and transverse sections. A total of 90 sections were collected (60 horizontal and 30 transverse sections) from each animal. The 5-HT-labeled fibers were examined in the grafted region and ventral horn of the spinal cord by counting the number of 5-HT fibers under high magnification of fluorescent or light microscope. Ninety sections per rat were counted, and the counts for all rats in each group were averaged.

To process the immunostaining of the PNG–spinal cord area, the horizontal sections were blocked in 3% normal horse serum with 0.25% Triton X-100 in PBS for 1 h. After blocking, the sections were exposed to anti-5-HT polyclonal antibody (1:1500 dilution; DiaSorin, Stillwater, MN, USA) and anti-glial fibrillary acidic protein (GFAP) for astrocytes (1:500; DakoCytomation, Carpinteria, CA, USA) and then incubated overnight at 4 °C. The sections then were washed, incubated with fluorescein-conjugated secondary antibody for 90 min, and coverslipped with Fluoromount mounting medium (Biomedical Specialties). All horizontal sections were examined under a microscope with fluorescent light. To process the 5-HT immunohistochemistry in the lumbar area, transverse sections were blocked in 3% normal horse serum with 0.25% Triton X-100 in PBS for 1 h. After blocking, the sections were exposed to anti-serotonin (5-HT) polyclonal antibody (1:1500 dilution; DiaSorin, Stillwater, MN, USA) and incubated overnight at 4 °C. After 3 rinses in PBS, the sections were exposed to a biotinylated secondary antibody (1:200; Vector, Burlingame, CA, USA) followed by the ABC Elite kit (Vector, Burlingame, CA, USA) for 1 h each. The reaction was visualized by treatment with 0.02% 3,3′-diaminobenzidine with 0.001% H_2O_2 in Tris–saline for 2–6 min, and the sections were then examined using a light microscope.

Statistical analyses

All data are reported as mean±standard error of the mean. A two-way (group × time) ANOVA followed by Tukey's post-ANOVA test were used to determine significant differences between groups. Linear regressions were performed using SigmaStat® statistical software. significant differences were determined at $P<0.05$.

Results

PNG with αFGF improves step cycle trajectories in spinal cord-transected rats

The data recorded by the robotic linkages during step testing were used for the kinematics analyses to examine the step cycle trajectories in all groups (Fig. 1). The ST and repaired groups were compared to determine the effects of PNG and αFGF on locomotor ability. Short, inconsistent steps were observed in the ST group (Fig. 1B), and no differences were observed between 3 and 6 months after surgery (data not shown). In contrast, longer, more consistent step cycle trajectories were observed in the repaired than the ST group (compare Figs. 1B and D). There was, however, no difference in the performance of the repaired between 3 and 6 months after surgery (data not shown). Furthermore, although several large amplitude steps were recorded in the repaired group, the mean trajectory was small compared to that in the sham group (compare Figs. 1A and D). The ST (Fig. 1B) and repaired (Fig. 1D) groups were used for comparison with the ST–step-trained (Fig. 1C) and repaired–step-trained (Fig. 1E), respectively, to determine the effect of step training. There were no significant differences for any of these comparisons at either 3 or 6 months after surgery.

αFGF with PNG, but not step training, improves step cycle kinematics features in spinal cord-transected rats

The kinematics data were obtained during a step test at 3 and 6 months after surgery. The repaired–step-trained group had a higher number of steps than the ST–step-trained group at both time points (Fig. 2A). The repaired group also had a higher step number than the ST–step-trained group at 6 months after surgery. The cycle duration was lower in all ST groups compared to the sham group except for the repaired group at both time points (Fig. 2B). In addition, the cycle period was longer in both repaired groups compared to the ST–step-trained group at 6 months. Stance duration was shorter in all spinal cord-transected groups compared to the sham group (Fig. 2C). Both repaired groups had longer stance durations than the ST–step-trained group. In contrast, the only difference from the sham group in swing duration was a shorter duration for the ST–step-trained group at 6 months (Fig. 2D). The repaired group also had longer swing durations that the ST–step-trained group at both time points.

Mean step length (Fig. 2E), maximum step length (Fig. 2F), mean step height (Fig. 2G), and maximum step height (Fig. 2H) were lower in all ST groups compared to the sham group at both time points. One or both repaired groups had higher values than the ST–step-trained group for each of these parameters at both time points.

The only difference for XPCA (Fig. 2I), YPCA (Fig. 2J), and FFT (Fig. 2K) at 3 months was a higher YPCA for the repaired–step-trained than ST–step-trained group. At 6 months after surgery, the XPCA and YPCA were higher and the FFT lower in both repaired groups compared to the ST–step-trained group. In addition, the ST–step-trained group had a higher FFT than the sham group. There were no significant within group differences for any parameter across time points.

PNG with αFGF plus step training improves the coordination of the soleus and TA EMG activity and the hindlimb kinematics in spinal cord-transected rats

The kinematics analyses show small excursions at all joints and dragging of the distal joint during the swing phase in the ST–step-trained rats, whereas the repaired–step-trained rats show more consistent stepping with larger joint excursions, particularly at the ankle and knee joints, and substantial foot clearance during the swing phase (Fig. 3A). The repaired–step-trained group showed more robust EMG activity of the soleus and TA muscles during

stepping on the treadmill than the ST–step-trained group (Figs. 3B and C). Furthermore, the activation of the soleus and TA was more coordinated (alternating activity shown as joint probability distributions) in the repaired–step-trained than the ST–step-trained group (Fig. 3D).

Regrowth of serotonin fibers after spinal cord transection with PNG and αFGF treatment

One of the goals of the current study was to investigate the contribution or axonal regeneration to motor recovery after a complete spinal cord transection. One of the major supraspinal projections for motor control, i.e., 5-HT fibers, was used to investigate the presence of regenerated descending fibers. Four rats from each group were included in this analysis. No 5-HT fibers were observed at or below the injured site in both the ST and ST–step-trained groups (Figs. 4D–F and 5B–C). Most of the 5-HT fibers were found immediately rostral to the injured/scar area. In contrast, repaired and repaired–step-trained rats had 5-HT-positive fibers at and below the graft site (Figs. 4A–C and 5D–G). The total numbers of 5-HT fibers at the graft site of the repaired and repaired–step-trained rats were 218.4 ±28.3 and 283.5 ± 35.8, respectively. In addition, the numbers of 5-HT fibers in the spinal cord within 1 mm below the graft of the repaired and repaired–step-trained rats were 85.7 ± 9.2 and 79 ± 14.6, respectively. The density (within or caudal to the graft site) of 5-HT fibers was not different between the two groups ($P>0.05$).

Several 5-HT fibers also were found in the ventral horn area of the lumbar spinal cord from both the repaired (Figs. 5D and F; mean 18.7±2.3) and repaired–step-trained (Figs. 5E and G; mean 21.5±4.9) groups. The 5-HT fiber density in the lumbar ventral horn was not different between the two groups ($P>0.05$). The 5-HT fibers in the repaired groups (Figs. 5F and G) were long compared to the dot structure observed in the sham group (Fig. 5A), even in cross sections.

Relationships among 5-HT immunoreactivity, stepping recovery, and muscle activation

There was a significant relationship ($r = 0.773$) between the improvement in stepping performance (step number) and the number of 5-HT-positive fibers in the ventral horn of the lumbar spinal cord of repaired and repaired–step-trained rats (Fig. 6). In addition, the mean EMG amplitudes of the both muscles were higher in the repaired–step-trained than ST–step-trained rats (Fig. 3C).

Discussion

There are three primary observations in the present study. First, the combination of a peripheral nerve fiber implant and the administration of αFGF at the site of a complete spinal cord transection at T8 resulted in a greater level of recovery of hindlimb locomotion than observed in spinal rats not receiving the repair procedure. This difference was reflected in a battery of quantitative measures of locomotion. Second, the level of recovery of locomotor ability among the rats receiving the repair procedures was not affected by step training. Third, while there was a significant relationship between stepping performance and the presence of 5-HT fibers caudal to the lesion, it remains unclear as to what degree the improved motor function can be attributed to the presence of these fibers.

PNG plus αFGF facilitate stepping performance and kinematics features in spinal rats

Adult rats, unlike adult cats (Barbeau and Rossignol, 1987; de Leon et al., 1998; Lovely et al., 1990), show little recovery of locomotor ability spontaneously or even with step training after a complete midthoracic spinal cord transection. Previous studies have suggested that PNG plus αFGF implants improve hindlimb motor function in rats after a complete spinal cord transection based on observations made in an open field assessment (Cheng et al.,

1996; Lee et al., 2002b; Lee et al, 2004a; Tsai et al., 2005). In the present study, we used a robotic device to train stepping on a treadmill and to provide a comprehensive set of quantitative parameters that characterize multiple aspects of locomotor recovery. The rats receiving the repair surgery completed more steps and most of the kinematics measures of locomotion were improved compared to those not receiving the nerve repair by 3 months after surgery. In addition, a higher percentage of animals receiving the repair procedures took more steps than the group average at 6 compared to 3 months (57% vs. 42%).

Step training with PNG plus αFGF enhances weight support but not the kinematics features of stepping

The efficacy of step training on a treadmill in the recovery of locomotor ability of spinal cats has been clearly demonstrated previously (Edgerton et al., 2004; Rossignol, 2000). It also has been shown that neonatal rats with a complete spinal cord transection can achieve weight-supporting stepping if they are trained to step on a treadmill (Kubasak et al., 2005). There is no evidence, however, showing an effect of step training alone in the recovery of stepping in adult rats with a complete spinal cord transection. Similarly, we observed no consistent effect of step training at either 3 or 6 months after lesion. Although there was no positive effect on the stepping performance and kinematics features, the two repaired–step-trained rats demonstrated a greater level of coordination between the flexor and extensor motor pools and higher EMG amplitudes than the ST–step-trained rats.

What factors may be relevant to the absence of an effect of step training in either the ST or repaired rats in the present study? We have consistently observed that it is necessary for the spinal circuitry to have a critical level of excitability if it is to respond to the proprioceptive input associated with load-bearing stepping (Edgerton et al., 1997, 2004, 2006). In rats receiving a complete spinal cord transection as adults, we have shown that this critical level of excitability is not reached with step training alone but can be attained using either epidural stimulation and/or pharmacological interventions to facilitate the step training (Edgerton et al., 2004, 2006). Thus, in the present study, one possibility is that this critical level of excitability was not present even in the rats that received the repair intervention. It also should be noted that there are other cases where no training effects were observed in models of spinal cord injury involving adult rats (Maier et al., 2009; Garcia-Alias et al., 2008; Ichiyama et al., 2009).

PNG plus αFGF promotes the regrowth of descending fibers

Several studies have reported that PNG plus αFGF can facilitate axonal regeneration either through a complete spinal cord lesion and some functional recovery based on BBB scores (Cheng et al., 1996; Lee et al., 2002b; Lee et al., 2004a; Tsai et al., 2005) or in vitro model (Lee et al., 2002a). In contrast, treatment with αFGF only did not show nerve fibers growing into the distal end of the spinal cord or any improvement in BBB scores. This repair strategy also has been reported to provide a neuroprotective effect (Lee et al., 2006) perhaps by attenuating oxidative stress in the spinal cord (Lee et al., 2004b). In the current study, 5-HT fibers, one of the major descending fiber populations that can play an important role in controlling locomotion, were identified within the graft as well as in the ventral horn caudal to the lesion site in the repaired groups. Since there were no 5-HT-positive fibers found between the two stumps of the spinal cord in either the ST or ST–step-trained rats, the 5-HT fibers crossing the transection site seems to have been facilitated by the PNG repair intervention.

Numerous studies have demonstrated an important effect of serotonergic agonists, e.g., quipazine, in facilitating locomotion in rats with a complete spinal cord transection (Antri et al., 2002; de Leon and Acosta, 2006; Ichiyama et al., 2008; Courtine et al., 2009). While

there is no proof for a causal relationship, we found a high correlation between the presence of 5-HT fibers in the lumbar ventral horn and the number of steps that could be generated in a given time period. In a previous study, we also demonstrated motor-evoked potentials in rats that had received PNG plus αFGF treatment (Lee et al., 2002b). In addition to the high correlation between the number of 5-HT fibers observed below the transection site and the number of steps performed, further studies are needed to provide more direct evidence of the contribution of axonal regeneration to motor recovery, e.g., determine any colocalization of synaptic markers with regenerated fibers and examine the effects of depletion of 5-HT at the nerve terminals below the lesion using pharmacological application on stepping performance. All of the results, however, are consistent with, but not conclusive, that descending pathways could have contributed to the recovery of some locomotor function in PNG-treated rats after the spinal cord injury. It should be pointed out, however, that the presence of 5-HT fibers in the rat spinal cord below a complete transection may not be a reliable indicator of regeneration (Newton et al., 1986; Newton and Hamill, 1988; Kubasak et al., 2008; Takeoka et al., 2009). For example, occasional 5-HT-labeled interneurons immediately dorsal or dorsolateral to the central canal have been observed in the caudal stump of the spinal cord after a complete transection with no signs of regeneration through the lesion site (Takeoka et al., 2009).

Conclusion

PNG and αFGF treatments, with or without step training, resulted in a greater improvement in stepping performance after a complete midthoracic spinal cord transection. In addition, repaired–step-trained rats showed the highest amount of improvement in the levels of EMG activity and flexor–extensor coordination in the hindlimbs after the spinal cord injury. The appearance of more 5-HT fibers below the lesion in the repaired than the ST groups and the significant correlation between the number of 5-HT fibers and stepping performance in the repaired groups are consistent with the improvement in locomotor function being attributable, at least in part, to the proliferation of 5-HT fibers in this preparation.

Acknowledgments

We thank Drs. Xioming Deng and Ximing Xiong for their assistance in training the spinal rats to step. This work was supported by the US Department of Veteran Affairs, Rehabilitation Research and Development Service (Merit Review B2902R) and NIH grant NS16333 (V.R.E.).

References

Antri M, Orsal D, Barthe JY. Locomotor recovery in the chronic spinal rat: effects of long-term treatment with a 5-HT_2 agonist. Eur. J. Neurosci 2002;16:467–476. [PubMed: 12193190]

Barbeau H, Rossignol S. Recovery of locomotion after chronic spinalization in the adult cat. Brain Res 1987;412:84–95. [PubMed: 3607464]

Cha J, Heng C, Reinkensmeyer DJ, Roy RR, Edgerton VR, De Leon RD. Locomotor ability in spinal rats is dependent on the amount of activity imposed on the hindlimbs during treadmill training. J. Neurotrauma 2007;24:1000–1012. [PubMed: 17600516]

Cheng H, Cao Y, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science 1996;273:510–513. [PubMed: 8662542]

Courtine G, Gerasimenko Y, van den Brand R, Yew A, Musienko P, Zhong H, Song B, Ao Y, Ichiyama RM, Lavrov I, Roy RR, Sofroniew MV, Edgerton VR. Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. Nat. Neurosci 2009;12:1333–1342. [PubMed: 19767747]

de Leon RD, Acosta CN. Effect of robotic-assisted treadmill training and chronic quipazine treatment on hindlimb stepping in spinally transected rats. J. Neurotrauma 2006;23:1147–1163. [PubMed: 16866627]

de Leon RD, Hodgson JA, Roy RR, Edgerton VR. Locomotor capacity attributable to step training versus spontaneous recovery following spinalization in cats. J. Neurophysiol 1998;79:1329–1340. [PubMed: 9497414]

de Leon RD, Hodgson JA, Roy RR, Edgerton VR. Hindlimb locomotor and postural training modulates glycinergic inhibition in the spinal cord of the adult spinal cat. J. Neurophysiol 1999;82:359–369. [PubMed: 10400964]

Edgerton, VR.; de Leon, RD.; Tillakaratne, N.; Recktenwald, MR.; Hodgson, JA.; Roy, RR. Advances in Neurology: Neuronal Regeneration, Reorganization and Repair. Lippincott-Raven Publishers; Philadelphia: 1997. Use-dependent plasticity in spinal stepping and standing.; p. 233-247.

Edgerton VR, Tillakaratne NJ, Bigbee AJ, De leon RD, Roy RR. Plasticity of the spinal neural circuitry after injury. Annu. Rev. Neurosci 2004;27:145–167. [PubMed: 15217329]

Edgerton VR, Kim SJ, Ichiyama RM, Gerasimenko YP, Roy RR. Rehabilitative therapies after spinal cord injury. J. Neurotrauma 2006;23:560–570. [PubMed: 16629637]

Garcia-Alias G, Lin R, Akrimi SF, Story D, Bradbury EJ, Fawcett JW. Therapeutic time window for the aoolication of chondroitinase ABC after spinal cord injury. Exp. Neurol 2008;210:331–338. [PubMed: 18158149]

Ichiyama RM, Gerasimenko Y, Jindrich DL, Zhong H, Roy RR, Edgerton VR. Dose dependence of the 5-HT agonist quipazine in facilitating spinal stepping in the rat with epidural stimulation. Neurosci. Lett 2008;438:281–285. [PubMed: 18490105]

Ichiyama RM, Potuzak M, Balak M, Kalderon N, Edgerton VR. Enhanced motor function by training in spinal cord contused rats following radiation therapy. PLoS ONE 2009;4:e6862. [PubMed: 19718437]

Kubasak MD, Hedlund E, Roy RR, Carpenter EM, Edgerton VR, Phelps PE. L1 CAM expression is increased surrounding the lesion site in rats with complete spinal cord transection as neonates. Exp. Neurol 2005;194:363–375. [PubMed: 16022864]

Kubasak MD, Jindrich DL, Zhong H, Tokeoka A, McFarland KC, Muñoz-Quiles C, Roy RR, Edgerton VR, Ramón-Cueto A, Phelp PE. OEG implantation and step training enhance hindlimb-stepping ability in adult spinal transected rats. Brain 2008;131:264–276. [PubMed: 18056162]

Lee YS, Baratta J, Yu J, Lin VW, Robertson RT. AFGF promotes axonal growth in rat spinal cord organotypic slice co-cultures. J. Neurotrauma 2002a;19:357–367. [PubMed: 11939503]

Lee YS, Hsiao I, Lin VW. Peripheral nerve graft and aFGF restore partial hindlimb function in adult paraplegic rats. J. Neurotrauma 2002b;19:1203–1216. [PubMed: 12427329]

Lee YS, Lin CY, Robertson RT, Hsiao I, Lin VW. Motor recovery and anatomical evidence of axonal regrowth in spinal cord-repaired adult rats. J. Neuropathol. Exp. Neurol 2004a;63:233–245. [PubMed: 15055447]

Lee YS, Sindhu RK, Lin CY, Ehdaie A, Lin VW, Vaziri ND. Effects of nerve graft on nitric oxide synthase, NAD(P)H oxidase, and antioxidant enzymes in chronic spinal cord injury. Free Radic. Biol. Med 2004b;36:330–339. [PubMed: 15036352]

Lee YS, Lin CY, Robertson RT, Yu J, Deng XM, Hsiao I, Lin VW. Re-growth of catecholaminergic fibers and protection of cholinergic spinal cord neurons in spinal repaired rats. Eur. J. Neurosci 2006;23:693–702. [PubMed: 16487151]

Lee YS, Lin CY, Caiozzo VJ, Robertson RT, Yu J, Lin VW. Repair of spinal cord transection and its effects on muscle mass and single fiber myosin heavy chain isoform phenotype. J. Appl. Physiol 2007;103:1808–1814. [PubMed: 17717118]

Lovely RG, Gregor RJ, Roy RR, Edgerton VR. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. Brain Res 1990;514:206–218. [PubMed: 2357538]

Maier IC, Ichiyama RM, Courtine G, Schnell L, Lavrov I, Edgerton VR, Schwab ME. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. Brain 2009;132:1426–1440. [PubMed: 19372269]

Moshonkina T, Avelev V, Gerasimenko Y, Mathur R, Bijlani RL. Treadmill training accelerates restoration of locomotion after complete spinal cord transection in the rat. Indian J. Physiol. Pharmacol 2002;46:499–503. [PubMed: 12683228]

Newton BW, Hamill RW. The morphology and distribution of rat serotoninergic intraspinal neurons: an immunohistochemical study. Brain Res. Bull 1988;20:349–360. [PubMed: 3365563]

Newton BW, Maley BE, Hamill RW. Immunohistochemical demonstration of serotonin neurons in autonomic regions of the rat spinal cord. Brain Res 1986;376:155–163. [PubMed: 3719364]

Ramon-Cueto A, Cordero MI, Santos-Benito FF, Avila J. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. Neuron 2000;25:425–435. [PubMed: 10719896]

Rossignol S. Locomotion and its recovery after spinal injury. Curr. Opin. Neurobiol 2000;10:708–716. [PubMed: 11240279]

Roy RR, Hutchison DL, Pierotti DJ, Hodgson JA, Edgerton VR. EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. J. Appl. Physiol 1991;70:2522–2529. [PubMed: 1885445]

Sharma HS. Neurotrophic factors in combination: a possible new therapeutic strategy to influence pathophysiology of spinal cord injury and repair mechanisms. Curr. Pharm. Des 2007;13:1841–1874. [PubMed: 17584113]

Silver J, Miller JH. Regeneration beyond the glial scar. Nat. Rev. Neurosci 2004;5:146–156. [PubMed: 14735117]

Takeoka A, Kubasak MD, Zhong H, Roy RR, Phelps PE. Serotonergic innervation of the caudal spinal stump in rats after complete spinal transection: effect of olfactory ensheathing glia. J. Comp. Neurol 2009;515:664–676. [PubMed: 19496067]

Timoszyk WK, de Leon RD, London N, Joynes R, Minakata K, Roy RR, Edgerton VR, Reinkensmeyer DJ. Comparison of virtual and physical treadmill environments for training stepping after spinal cord injury. Robotica 2003;21:25–32.

Tsai EC, Krassioukov AV, Tator CH. Corticospinal regeneration into lumbar grey matter correlates with locomotor recovery after complete spinal cord transection and repair with peripheral nerve grafts, fibroblast growth factor 1, fibrin glue, and spinal fusion. J. Neuropathol. Exp. Neurol 2005;64:230–244. [PubMed: 15804055]

Xu XM, Guenard V, Kleitman N, Bunge MB. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. J. Comp. Neurol 1995;351:145–160. [PubMed: 7896937]

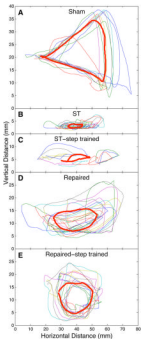

Fig. 1.

Ankle trajectories for 8-18 steps from a representative rat from the Sham, ST, ST-step trained, Repaired, and Repaired-step trained groups are shown. Data were collected during step tests at six months after spinal cord surgery. Thin lines, individual trajectories; thick line, mean trajectory.

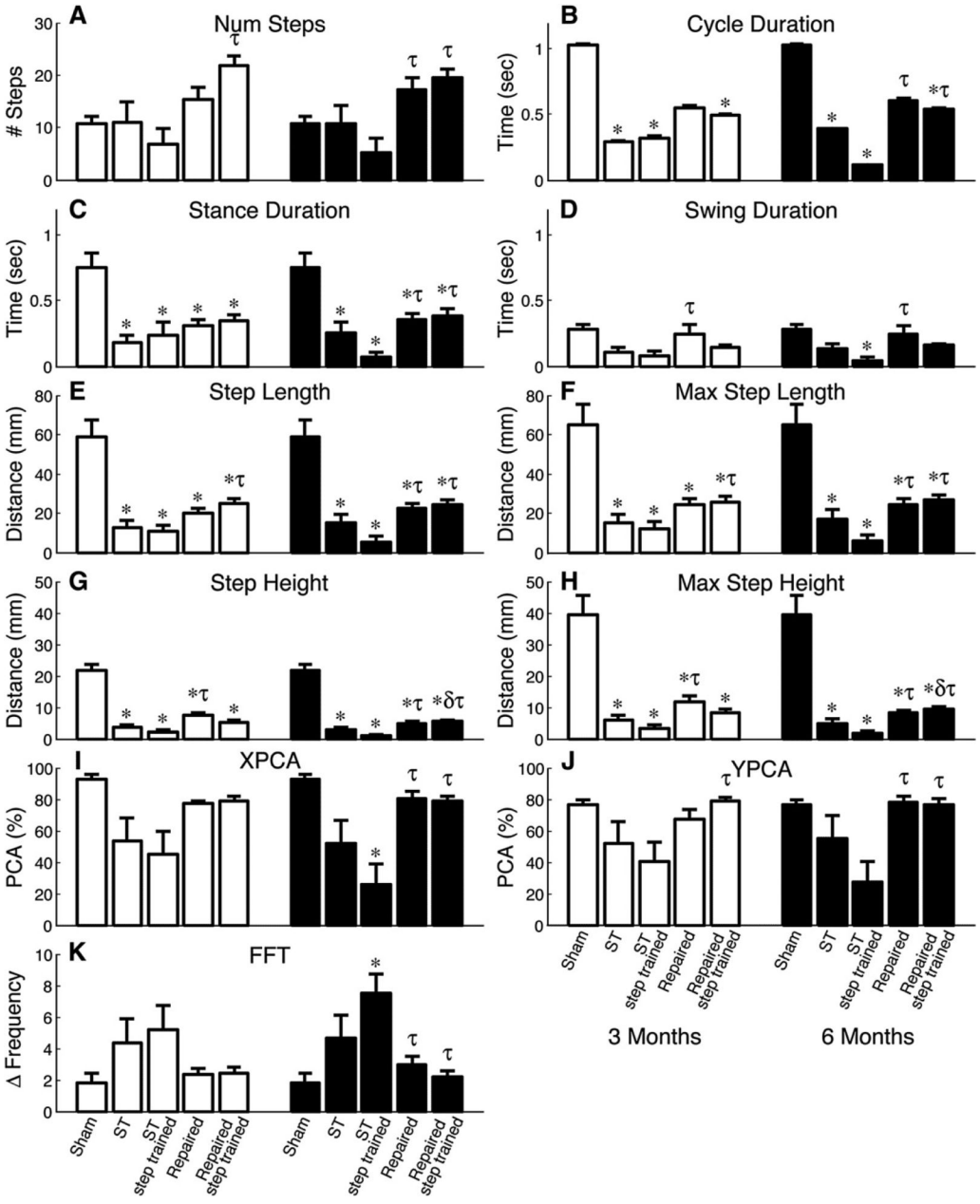

Fig. 2.

Comparisons of several kinematics features across groups and at 3 and 6 months after lesion are shown. There were no statistical differences between 3 and 6 months. Values are mean ± SEM. Max, maximum; XPCA, principal component analysis (PCA) of the hindlimb movement when moving in the horizontal direction; YPCA, PCA in the vertical direction; FFT, Fast Fourier Transform. *, δ, and t: significantly different from sham, ST, and ST-trained, respectively, at $P<0.05$.

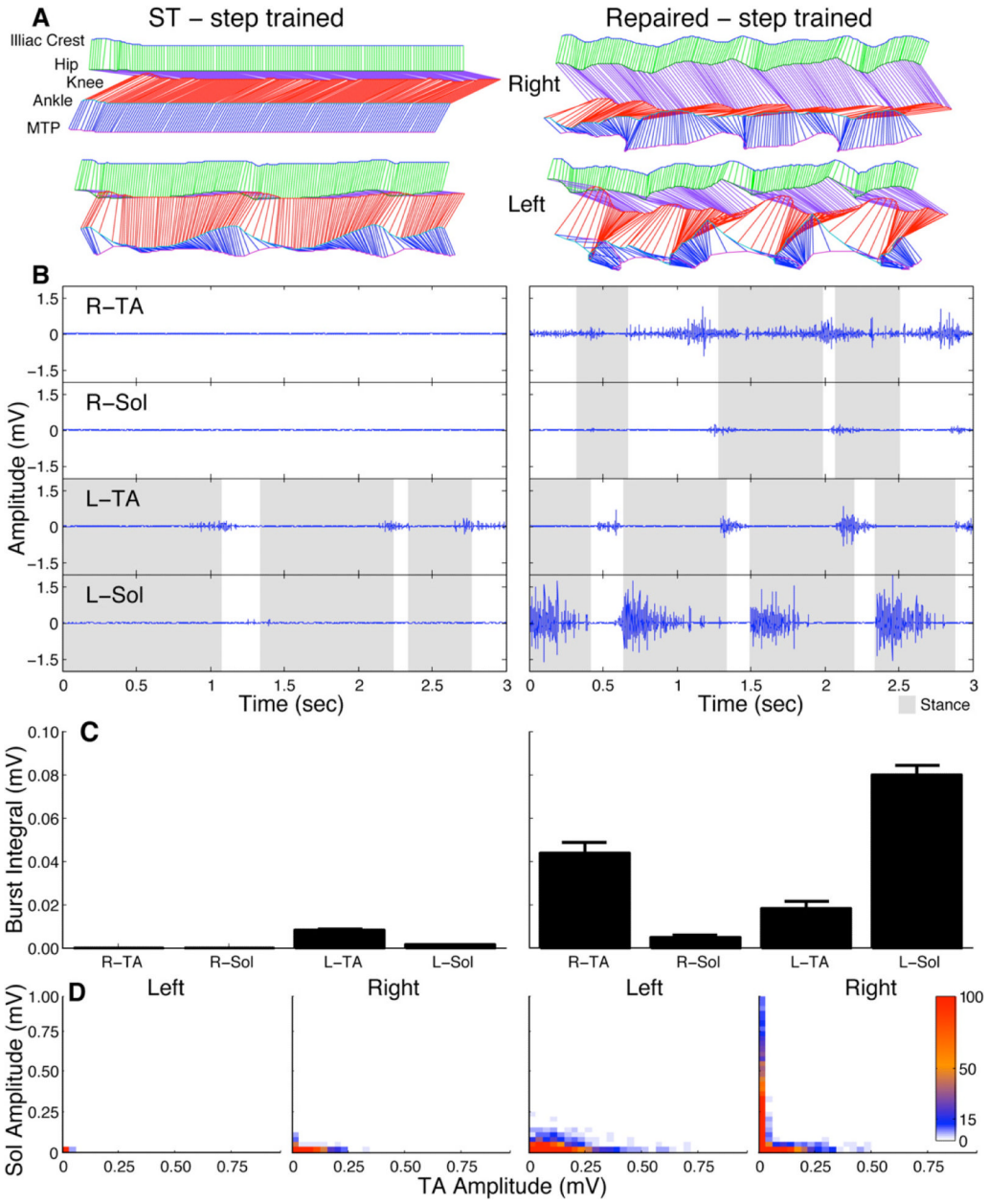

Fig. 3.

Stick diagrams (20 ms between sticks) of the hindlimbs during the best stepping performance (3 s) for the rat with the best performance from the ST–step-trained and repaired–step-trained groups are shown (A). Note that each leg shows a different pattern, with the right leg of the ST–step-trained rat dragging throughout the test period. The raw EMG signals from the Sol and TA bilaterally are shown for the ST–step-trained and repaired–step-trained rat (B). The shaded area highlights the stance phase of the step cycle. The mean integrated EMG (±SEM) for the same steps is shown in (C). Note that the EMG amplitudes for all muscles were higher in the repaired–step-trained than the ST–step-trained rat. Joint probability distributions of the EMG amplitudes for Sol and TA in both hindlimbs of the ST–step-trained and repaired–step-trained rat are shown (D). The color scale shown on the graphs is proportional to the incidence of a given pair of amplitudes.

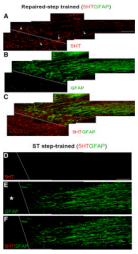

Fig. 4.

The confocal photomicrographs (A–C) from a horizontal section of a Repaired–step-trained rat demonstrates 5-HT fibers (red, arrow) growing from the PNG (GFAP (green) negative area) into the distal end of spinal cord (GFAP-positive area). (D–F) In ST–step-trained animals, there were no 5-HT fibers growing into the lesion site (*, GFAP-negative area) and reentering the distal end of the spinal cord tissue. (A, D) Staining for 5-HT. (B, E) Staining for GFAP. (C, F) Double staining for 5-HT and GFAP. The dash lines indicate the interface between the PNG and the distal end of the spinal cord tissue. Scale bars: 500 μm.

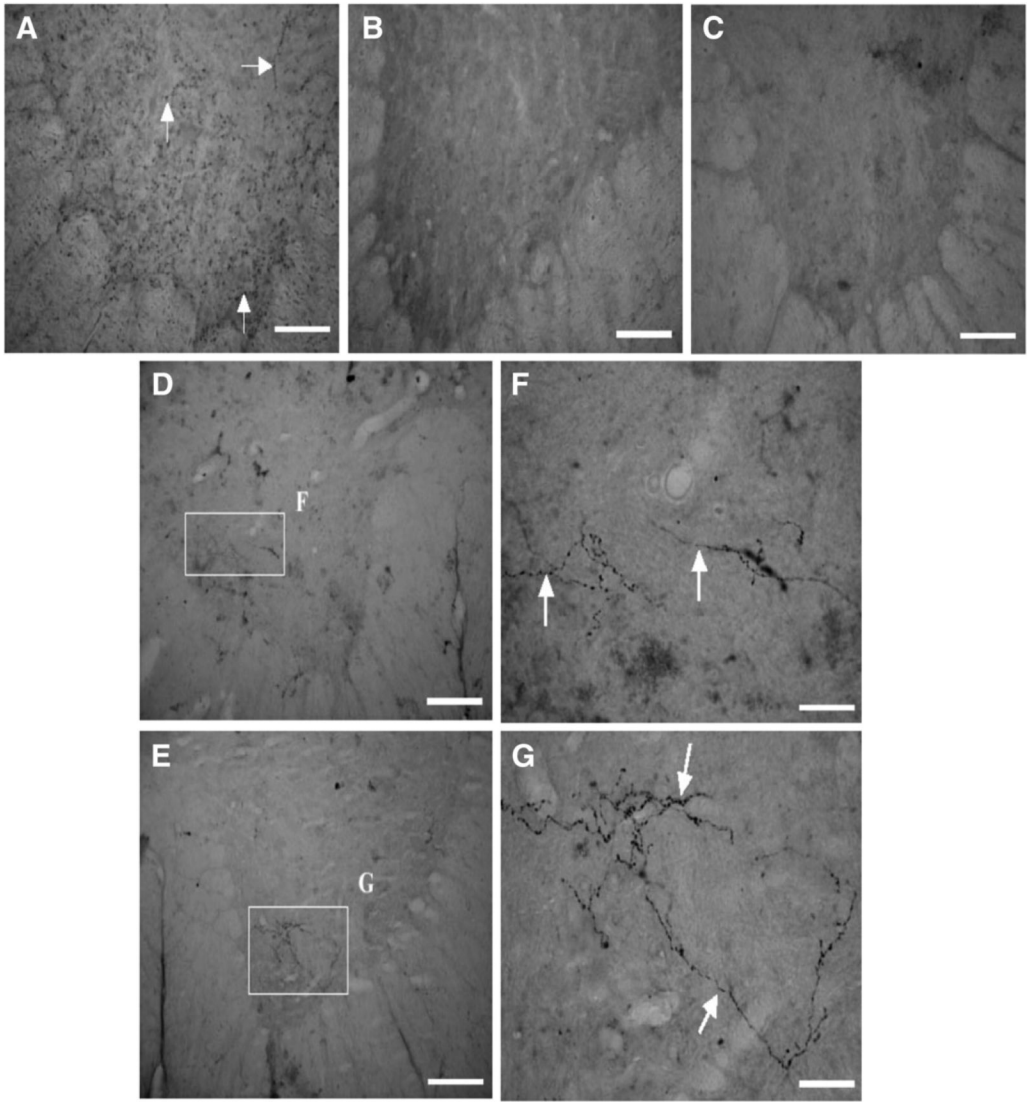

Fig. 5.

The photomicrographs from transverse sections show the immunoreactivity of 5-HT fibers in the ventral horn of the lumbar spinal cord from a representative rat from the sham (A), ST (B), ST–step-trained (C), repaired (D and F), and repaired–step-trained (E and G) groups. The boxed areas in (D) and (E) are expanded in (F) and (G), respectively. There were no 5-HT fibers observed in the ST and ST–step-trained rats. In contrast, 5-HT fibers were found in the sham, repaired, and repaired–step-trained rats. Arrows indicate 5-HT fibers. Scale bars: A–C=100 μm, D–E=200 μm, F–G=50 μm.

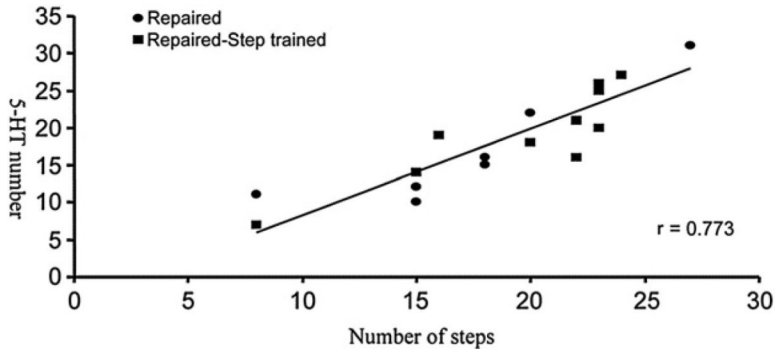

Fig. 6.

The relationship between the number of 5-HT fibers in the ventral horn of the lumbar spinal cord below the complete lesion and the number of steps performed during a 13-s test for the repaired and repaired–step-trained rats is shown ($r = 0.773$). Abscissa = number of steps; ordinate = number of 5-HT fibers.