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Review article

Effects of locomotor training on the functional recovery from the spinal cord injury

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Abstract

This mini-review surveys several representative rehabilitation studies using a treadmill or other methods of locomotor exercises in humans and experimental animals with spinal cord injury. The methods and effect of locomotor training employed in individual studies are explained and the importance of the sensory input and body weight loading in the stimulation of the central pattern generator is emphasized. The establishment of neural networks by regenerating and/or spared axons is the basis of locomotor improvement. Although regenerating axons are found within the lesion, it is difficult to demonstrate the development of new neural connections. Muscle activity is another important factor in recovery from spinal cord injury. Robotic trainings of rats on a treadmill is not considered suitable for a rehabilitation study, because the robotic movement of the hind limbs differs from natural quadrupedal walking. Clinically, driven gait orthosis is used effectively for locomotor training of patients with SCI.

Key words : treadmill, locomotor training, central pattern generator, neural network, muscle activity, robotic training, driven gait orthosis

Introduction

Spinal cord injury (SCI) is a devastating disorder resulting in the severe loss of locomotor as well as sensory functions below the level of injury. The problem of this disorder is that the improvement in locomotor function is very limited even with extensive rehabilitation therapy. Various types of treatment, including surgical intervention, cell transplantation and rehabilitation therapy, have been employed in order to improve locomotor functions in patients with SCI. However, a functional recovery sufficient for undertaking normal activity of daily life (ADL) is not common. Patients with a complete spinal cord injury are in many cases constrained to a wheel chair for life.

Clinically, the rehabilitation is an important therapy for the treatment of SCI. One of the major purposes of rehabilitation is to improve the locomotor function. In many studies, locomotor exercises are done on a treadmill. We have studied the effect of treadmill training on the locomotor behavior of rats with SCI. Rats were injured by contusion at the T8-9 level of the spinal cord, and the treadmill training was given for 4 weeks beginning at 1 week post-injury. We examined several parameters of locomotor function, tissue repair, and nerve regeneration in rats with SCI (Hayashibe et al, 2013). Their body weight was supported in a harness during the treadmill exercises. Body weight support training (BWST) using a harness is recognized as an effective in treadmill training. In addition to BWST, the animals received manual helps to perform plantar stepping, while their body weight was only partially unloaded.

In this mini-review, we survey several representative rehabilitation studies that used a treadmill or other methods for the locomotor exercises. Improvement of locomotor function is related to the activation of neural networks. Axonal regeneration, synaptic reconnections of regenerated and/or spared axons, and the stimulation of the central pattern generator (CTG) contribute to the activating neural networks in the spinal cord lesion. The muscle function above and below the level of injury is also a critical factor in the locomotor function. Dietz et al. (2006) emphasized the effects of treadmill training that activates the neural circuit in the injured spinal cord. Spinal cord circuits have both activity-dependent and injury-induced plasticity. Through locomotor training, spinal neuronal circuits become activated by the appropriate sensory inputs. Hip joint-related afferent inputs might be essential to generate a locomotor activity in patients. The fact that the timing of the pattern of the leg muscle electromyograph (EMG) activity in patients with SCI be similar to that found in healthy subjects is important. Dietz et al. state that "axonal regeneration over a short distance to the long propriospinal neuronal circuits might be sufficient to mediate locomotor function," and that "as little as 10% to 15% of functioning tract fibers may be sufficient to allow a basic function in individuals with initially complete SCI." They showed that the human spinal cord has the capacity to generate a locomotor pattern and a certain potential neural plasticity in patients with SCI. (In this mini-review, the direct citations of sentences from original papers are shown in quotation marks.)

Locomotor training in general

Treadmill training aims in part to facilitate the activity of the CPG in the spinal cord (Fig. 1). The CPG is not limited to local regions of the spinal cord, but is distributed throughout the spinal cord (Rybak et al. 2006). Grasso et al. (2004) state that locomotor responses elicited during the treadmill training in SCI patients might depend on a plastic redistribution of activity across most of the rostrocaudal extention of the spinal cord. Sensory inputs from the foot sole, extension of hip joint, and walking speed are considered to promote CPG activity. Knikou (2010) suggested in his review that the "CPG may rely heavily on sensory feedback and remnant descending inputs." Dobkin et al. (1995) reported that the treadmill training with the partial body weight support enhanced walking in patients with SCI. They showed that sensory inputs were important in the BWST training of patients, and that the hip extension at the end of stance was critical to induce hip flexion. As mentioned above, Diez et al. (2002) showed that the afferent input from hip joints and the input from load receptors play a crucial role in the regeneration of locomotor activity in the spinal cord of patients with complete para/tetraplegia.

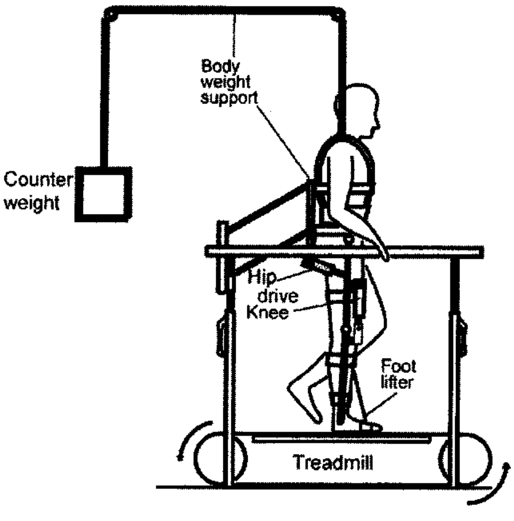

Fig. 1 Locomotion on the treadmill within a driven gait orthosis. (Dietz et al. 2002, with permission)

Goldshmit et al. (2008) carried out an extensive study on the effect of treadmill training on locomotor behavior and on axonal regeneration in mice. The spinal cord of mice was hemisectioned at T12 on the left side. Treadmill speed was 6~12 m/min, and training was done twice daily, 10 min each time. Training started at 1 week post-surgery. Animals were not body weight-supported. They were urged to walk on the treadmill by gentle tapping or by blowing of air onto their backs.

Behavioral ability was assessed using various techniques, including a modified BBB scale (Basso et al. 1995), horizontal grid walking, angle grid climbing, 2-mm-diameter rod grasping, kinematic gait analysis, and foot analysis (Kunkel-Bagden, et al. 1993).

The horizontal grid walking was performed to measure the accuracy of limb placement and the precision of motor control. "When the left hind limb paw protruded entirely through the grid with all toes and heel extending below the wire

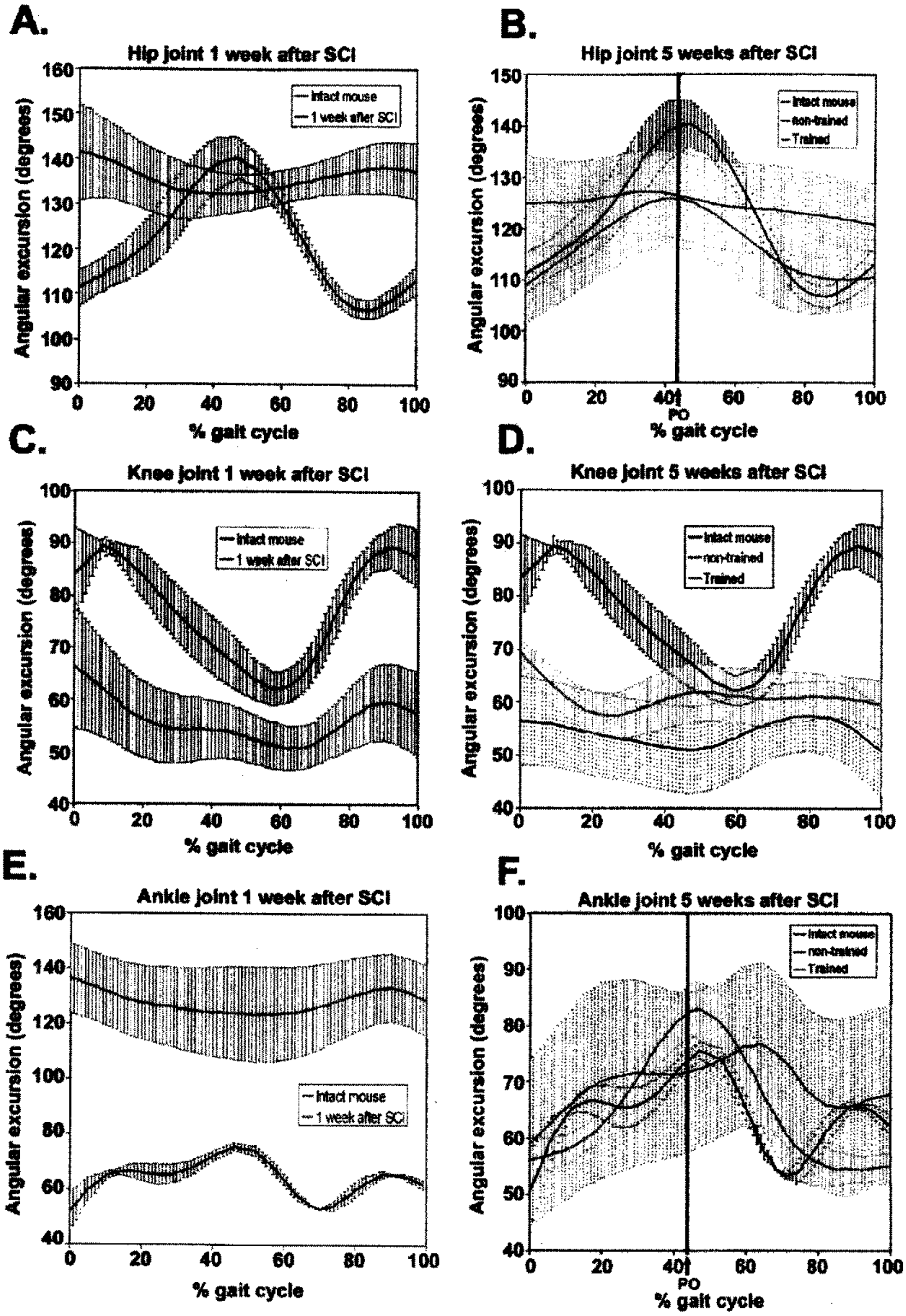

Fig. 2 Kinematic profile of individual joints following spinal cord injury (SCI). Average kinematic profile of angular excursions of the left hip (A, B), knee (C, D), and ankle (E, F) joints in mice 1 and 5 weeks after SCI in trained and untrained mice were compared to the intact mouse pattern over one complete step cycle. Left graphs (A, C, E) show results for 1 week after SCI, and the right graphs (B, D, F) show results for 5 weeks after SCI. At 1 week, all SCI mice dragged their hind limbs and all joint angles showed little change. At 5 weeks, the hip (B) and ankle (F) joint graphs showed that movement of these joints in the trained mice approximated the movement of the intact mouse. The point of the push off (PO) and the beginning of the swing phase is indicated by a black vertical line through the graph with an arrow at its base. The large error bars in panel F for the untrained mice reflects the large variation in foot posture, with mice showing foot dragging and some spasticity, particularly when placed on the treadmill. The knee joint (D) angle was greater in exercised than in untrained mice but showed little variation during the step cycle. Results are expressed as mean ± SD obtained from n=8 untrained and n=7 trained mice. (Goldshmit et al. 2008, with permission)

surface, this was counted as a misstep. The percentage of correct steps was calculated." Angled grid climbing was used to evaluate animals' locomotor activity under more strenuous conditions. "Mice were tested climbing up a wire grid at an angle of 75 from the horizontal in order to further evaluate." The criteria for a misstep here were the same as above. The ability to grasp a 2-mm-diameter rod was a test of the sensorimotor ability. "The hind limbs of the mice were lifted 2 cm from the tabletop whilst allowing the front limbs to remain in contact with the table. Grasping ability was tested by lightly touching the left foot pad with the rod and assessed using a scale of 0–4." The movement of joints in the hind limbs was evaluated by kinematic gait analysis. Markers were put on the iliac crest, femoral head (hip joint), knee joint, and ankle joint. The sagittal plane motion of the mice was recorded by a video camera, and analyzed using the software "Peak Motus Motion Measurement System" (Fig. 2). Foot analysis is the analysis of the stepping pattern. "Non-toxic ink was put on hind paws of mice. Mice were placed in a tunnel on the sheet of paper and allowed to walk." They measured the stride length between the successive foot prints on left or right side, and the angle of a line drawn between left and right prints to the line along the moving direction (Fig. 3). These measurements were valuable in the assessment of stepping patterns in the locomotor training study. In that study, they examined axonal outgrowth using an anterograde axonal tracer, the neural connections using immunohistochemical demonstration of synaptic markers (Fig. 4), and the degree of atrophy of gastrocnemius and soleus muscles using histological preparations (Fig. 5). The results are as follows: "Treadmill trained mice showed a significant improvement in use of their paretic hind limb after 4 weeks of exercise compared to untrained mice in an open field locomotor test, grid walking and climbing, and inter-limb coordination tests. Unlike untrained mice, exercised mice showed decreased muscle atrophy, increased axonal regrowth and collateral sprouting proximal to the lesion, with the maintenance of synaptic markers on motor neurons in the ventral horn." However, regarding axonal regeneration, there was no finding suggesting axonal regeneration into or across the lesion site. Therefore they concluded that the improved behavior may have been, at least in part, due to the enhanced neural activity above the lesion site

The importance of loading on the hind limbs was shown by Timoszyk et al. (2005). The hind limb loading determines the quantity and quality of stepping of SCI rats. On a treadmill, they examined the bipedal hind limb stepping ability of untrained and trained spinal cord-injured rats at different levels of body weight support, and showed that the stepping ability improved only at

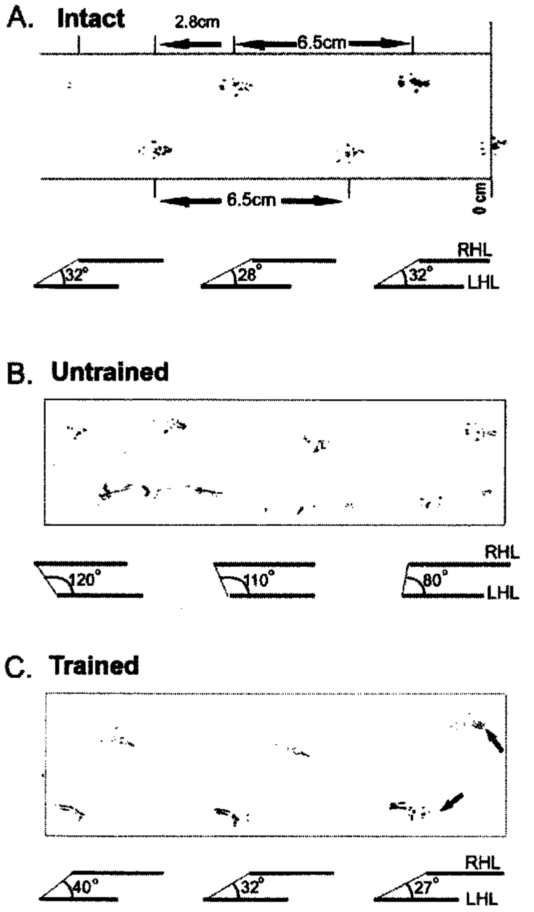

Fig. 3 Footprint analysis of hind limb coordination and stride length. Inter-hind limb coordination was analyzed by measurement of footprint of intact mice (n=14; A), untrained mice 5 weeks after SCI (n=7; B), and treadmill trained mice 5 weeks after SCI (n=7; C). The measurements of the distance between two successive steps in the left hind limb or in the right hind limb were measured from each footprint at the fifth toe print; as in the SCI mice, the footprint often did not include the heel area. (A) The footprints of an intact mouse show regular alteration between the right and the left hind limbs, as indicated by the angle of a line drawn between left and right prints, with a consistent stride length. (B) In untrained mice, 5 weeks after SCI, poor alteration between hind limbs was shown, with smeared left footprints due to left hind limb dragging and variable stride length. (C) Trained mice showed good alteration of their hind limbs, of a consistent angle, similar to the alteration in an intact mouse, and consistent stride length. However, the left pawprint was smeared at push off, and there was no heel contact, as indicated by arrows. (Goldshmit et al. 2008, with permission)

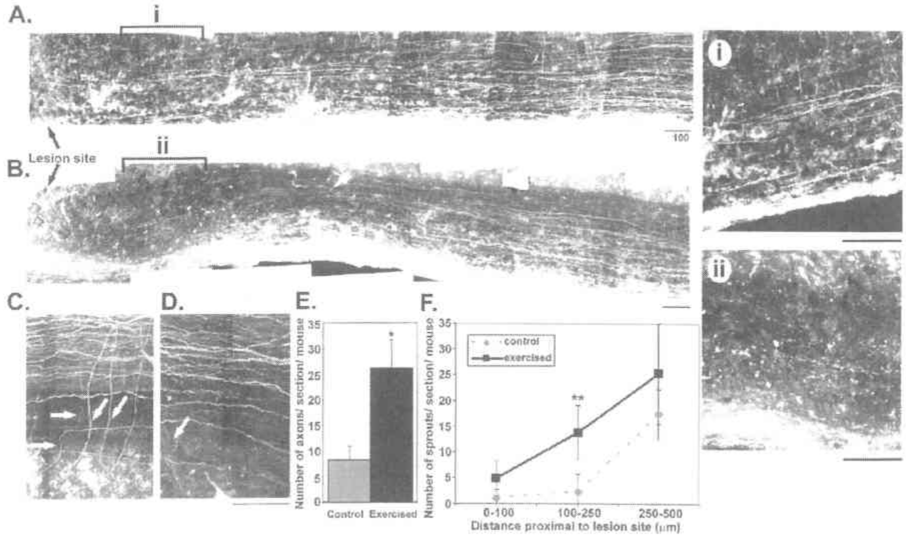

Fig. 4 Anterograde tracing of axonal growth proximal to the lesion site. Anterograde tracing and the confocal analysis of longitudinal spinal cord sections from lesioned treadmill-trained (n=8) and untrained (n=7) spinal cords 6 weeks after hemisection was used to assess the extent of axonal regeneration and sprouting. (A) Regenerative section indicating enhanced regrowth of axons and collateral sprouting towards the gray matter proximal to the lesion site in treadmill-trained mice (with higher power image in panel Ai). (B) Representative section from non-trained mice indicating fewer axons growing towards the lesion site than the exercised group, with higher power image in panel Bii. In both panels A and B, rostral is to the right and caudal to the left, and the lesion site is indicated by arrows. (C, D) Collateral sprouting proximal to the lesion site, with arrows pointing at example collateral sprouts entering into the grey matter in the trained (C) and untrained (D) mice. (E) Numbers of labeled axons reaching within 100 proximal to the lesion site were significantly greater in exercised mice compared to non-exercised controls. Results show mean ± SD ($*$ $p<0.0001$ using unpaired *t*-test). (F) Collateral sprouts were counted from the lesion edge to 500 μm proximal to the lesion site and indicated significantly more collateral sprouting in spinal cords from exercised mice compared to non-exercised controls, particularly between 100 and 250 μm (mean ± SD, $**$ $p<0.05$ using ANOVA with Bonferroni post-hoc test). Scale bar=100 μm (A, B, Ai, Bii), 50 μm (C, D). (Goldshmit et al. 2008, with permission)

the higher levels of weight-bearing.

Concerning the body weight bearing, Multon et al. (2003) performed an experiment to examine the effects of treadmill training with body weight support, reporting very promising results. For the spinal cord injury, a catheter was introduced into the subarachnoid space through a small hole in the dura mater. The balloon at the tip of the catheter was rapidly inflated with 20 μL of sterile water and left in place for 5 min. Treadmill speed was 5.8 cm/sec, and the training duration was 30 min/day (three sessions of 10 min separated by 5-min pauses). Animals were placed in an adjusted hanging harness to partially support body weight. Following the period of flaccid paraplegia, animals were stimulated by pinching the tail or rubbing the skin around the hip during the training session. "Mean BBB scores during 12 weeks were globally significantly greater in the treadmill-trained animals than in the control group, the benefit of training appearing as early as the 2nd week. At week 7, locomotor recovery reached a plateau in both groups, but remained superior in trained rats."

Sandrow-Feinberg et al. (2009) reported moderate effects of treadmill training. The spinal cord was injured using an impactor (Ohio State University Impactor) with a 1.6-mm-diameter stainless steel tip placed on the right side at the C4 spinal cord level. The impacting force was 200 Kdyne, causing a depression of 1600–1800 μm depth on the spinal cord surface. Treadmill speed was 5～14 m/min, without body weight support. The locomotor behavior was assessed using the open field locomotion BBB, a grid-walk (sensorimotor) test, a grip strength (motor) test, and a wheel walking test. In the grip strength test, the animal was urged to grip the metal bars attached to the force transducer with the affected forepaw. Once the grip was secured, the animal was slowly pulled horizontally from the bar. The transducer recorded the force at the point of grip release.

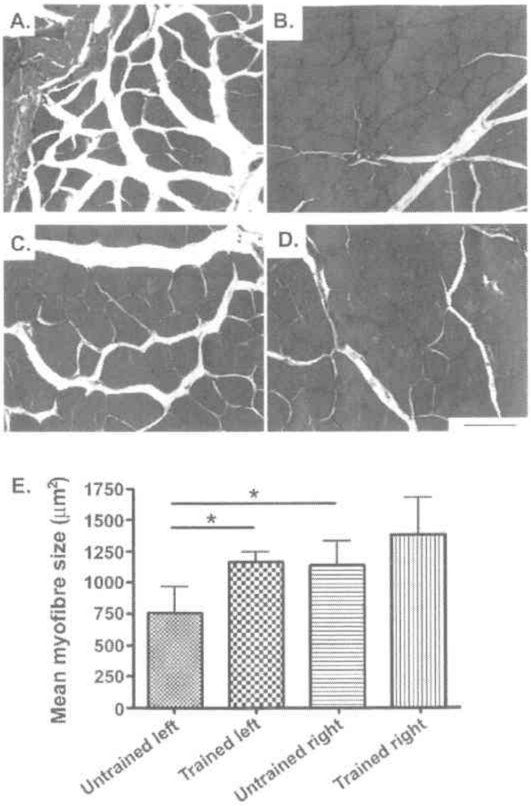

Fig. 5 Muscle atrophy is decreased in exercised mice. The effect of exercise in reducing muscle atrophy following SCI was analyzed by examination of Masson's trichrome stained gastrocnemius-soleus muscle from treadmill-trained (n=4) and untrained (n=4) mice 6 weeks after SCI. In the left muscle from untrained mice (A), there was significant atrophy, in comparison to either their right hind limb muscle (B), or the left (C) or the right (D) hind limb gastrocnemius-soleus muscle of treadmill-trained mice. (E) Mean myofiber area size for gastrocnemius-soleus muscle in trained and untrained mice of their right hind limb or left hind limb. Results show mean ± SD (unpaired *t*-test, $*p<0.05$, n=4 untrained and n=4 trained mice). Scale bar =50 μm (A-D). (Goldshmit et al. 2008, with permission)

They describe the results as follows: "The forelimb locomotor scale indicated a significant benefit of exercise during weeks 2-4. The BBB showed no changes with exercise at the end of the 8-week period, however, the hind limb grid performance was improved during weeks 2-4. The lesion size was not affected by exercises, but the presence of phagocytotic and reactive glial cells was reduced with the exercise as an intervention."

Battistuzzo et al. (2012) published an extensive review of treadmill covering 41 papers selected out of 362 from Medline. This review is helpful in planning a rehabilitation study with treadmill training. "The adult female rat was the most widely used animal model. The majority of studies (73%) reported that the exercise training had a positive effect on some aspect of locomotor recovery. For the incomplete SCI model, the contusion was the most frequently employed method of lesion induction, and the degree of recovery depended on injury severity. Positive outcomes were associated with training regimens that involved a partial weight-bearing activity, commenced within a critical period of 1-2 weeks after the SCI and maintained training for at least 8 weeks. Only 15% of the studies had high methodological quality. They recommended that future studies include control groups, randomize animals to groups, conduct blinded assessments, report the extent of the SCI lesion, and report sample size calculations." The explanations of individual studies should include animal strain, number of animals, ages at SCI, types of injury, duration of training, and methods of locomotor analysis, of randomization, and of data analysis.

On the other hand, there are a few studies reporting negative results of treadmill training. Fouad et al. (2000) reported rather negative findings on effects of treadmill training. In this study, the spinal cord was injured over 65% by transection at the dorsal side, interrupting the cerebrospinal and rubrospinal tracts. Treadmill speed was 7～10.5 m/min, without body weight support. The stepping of the hind limbs was elicited by manual stimulation of the perineum. "During the course of 5 weeks after the injury, a substantial amount of recovery occurred in the treadmill trained as well as the untrained rats. If compared to the control injured rats, which showed a high level of spontaneous hind limb movement at 7-14 days post lesion, no additional beneficial effect of a 5-week daily treadmill training on the locomotor outcome could be detected in the trained group. The only change observed was a slightly larger exploratory activity of the trained rats. It is probable that the spared ventral-lateral fibers allowed the spontaneous recovery and self-training to occur to such an extent that the systematic treadmill training did not provide an additional improvement."

Similarly, Moraska et al. (2000) warned of the negative effects of treadmill training. They trained normal uninjured rats. Rats showed stress reactions. "Forced treadmill running produced both positive and negative physiological adaptations. Indicative of the positive training adaptation, exercised male rats had a decrease in body weight gain and an increase in muscle citrate synthase activity compared with the sedentary control. In contrast, the treadmill running also resulted in the potentially negative adaptation of adrenal hypertrophy, thymic involution, decreased serum corticosteroid binding globulin, elevated lymphocyte nitric concentration, suppressed lymphocyte proliferation, and suppressed antigen specific IgM. Such alterations in neuroendocrine tissues and immune responses are commonly associated with chronic stress. Researches employing forced activity need to be aware that this type of exercise procedure also produces physiological adaptations indicative of chronic stress and that these changes could potentially impact other measures of interest."

It is interesting to compare swimming training with the treadmill training of SCI rats. Magnuson et al. (2009) reported the effect of swimming training on SCI rats. Swimming does not require weight support by the hind limbs, so that swimming training differs from the treadmill training. Their conclusion is as follows: "These data suggest that the activity pattern of swimming is hardwired in the rat spinal cord. After spinal cord injury, repetition is sufficient to bring about significant improvements in the pattern of hind limb movement but does not improve the forces generated, leaving the animals with persistent deficits. These data support the concept that the force (load) and pattern (recruitment) generation are independent of each other and may have to be managed together with respect to post-injury rehabilitation." Swimming training is related to the activation of the CPG, but not related to the force generation during locomotion. The force generation occurs in the treadmill training.

Nerve regeneration and neural circuit activation

Locomotor training is expected to enhance nerve regeneration in the spinal cord lesion. Many studies show findings indicating the occurrence of axonal regeneration within the lesion. However, it is difficult to show formation of synaptic connections by regenerating axons within and around the lesion. The current experimental techniques are insufficient to demonstrate the establishment of functionally effective neural networks.

Goldshmit et al. (2008) showed an increased axonal regrowth and collateral sprouting proximal to the lesion by demonstrating the existence of synaptic markers on motor neurons in the ventral horn (Fig. 4). However, they reported that there was no finding suggesting axonal regeneration into or across the lesion. They considered that the treadmill training enhanced the activity of neural circuits in the nerve tissues spared from the injury in SCI. The study by Kloos et al. (2005) supports this thought. They showed that the amount of white matter spared after SCI influences on the mode of functional recovery. The spinal cord was contusion injured at T8 with an electromagnetic SCI device developed at Ohio State University equipped with 1.6-mm-diameter probe. "The probe was slowly lowered onto the dorsal surface of the exposed spinal cord until a force transducer registered 3 Kdyne. On the next 25 msec, the probe vertically displaced the spinal cord surface by approximately 1.0 mm and then rapidly retracted." Seven SCI grades were produced according to the rates of compression on the spinal cord (displacement levels: 0.3, 0.5, 0.7, 0.9, 1.1, 1.25, and 1.3 mm in depth). "Locomotor and sensory recovery after contusive SCI gradations resulted in three locomotor performance levels measured with BBB. Normal paw position was most susceptible to SCI requiring 90% WMS (white matter sparing), while consistent plantar stepping was least susceptible depending on 10% WMS. Lesion severity correlated to WMS ($\gamma^2=0.96$) and to BBB ($\gamma^2=0.87$) by nonlinear polynomial regressions."

Several molecules have been proposed to enhance axonal regeneration in the SCI. Oh et al. (2009) reported that "the treadmill training for 2-4 weeks significantly improved behavioral functions of rats as assessed by the BBB scale," and that "Erk1/2 in the nervous system might be an important mediator for transmitting signals from the injury site to the cell body, being involved in enhanced outgrowth of cerebrospinal tract axons." Nerve regeneration is so complex an event that numerous molecules might be involved in the promotion of axonal regeneration. It is unlikely that spinal cord regeneration is dependent on any one specific molecule.

Non-neuronal components are also related to functional improvement of SCI. Foret et al. (2010) reported the ependymal cell reactions to spinal cord injury followed by treadmill training. The spinal cord was injured using an arterial embolectomy catheter, which was inserted into the epidural space at the T10 level and was inflated with a liquid of the volume of 15 μL for 5 min. "Treadmill training was performed on a custom-built device. Treadmill speed was 3.5 m/min for injured rats and 7 m/min for uninjured rats. Injured rats were suspended in a harness to allow locomotion during the treadmill training, and their hind limbs mobilized manually to initiate stepping." The results are as follows: "the exercise improved functional recovery and autonomous micturition, maintained nestin expression in both injured and uninjured spinal cords, with a positive correlation between the locomotor recovery and the number of nestin-positive cells. All ependymal cells of the central canal are Sox-2 positive. With the lesion, Sox-2 expression increased transiently, while the number of nestin-positive ependymal cells increased with the concomitant enhancement of proliferation." Although there was no evidence indicating that proliferation of ependymal cells was directly involved in functional improvement of rats with SCI, this study showed the importance of ependymal cells of the spinal cord central canal in the study of SCI. Ependymal cells are considered to be potential stem cells in the central nervous system. Ependymal cell behavior following SCI should be studied in more detail in the future.

Treadmill and muscle regeneration

In addition to neural connections within the spinal cord, muscle functions are also important in locomotor recovery from SCI. Stevens et al. (2006), Liu et al. (2010) and Jaramanan et al. (2012) examined the soleus muscle regenerative response in the treadmill training of rats. Spinal cord contusion was performed at T8 by dropping a 10-g weight from the height of 2.5 cm. Treadmill speed was 11 m/min, and body weight support was provided manually. Animals were given antibiotics, and anti-pain and anti-inflammatory drugs. A lactose-Ringer (5 ml) was injected subcutaneously after surgery. Manual treadmill training began 1 week after SCI. Two muscles were used for analysis: plantar flexor soleus muscle (slow-twitch muscle), and dorsiflexor tibialis anterior muscle (fast-twitch muscle). The expression of insulin-like growth factor-1, Pax 7 (a marker of satellite cell activation), myogenin and embryonic myosin were immunohistochemically examined. BBB scores at the beginning were 3-7 points to ensure a relatively homogenous animal study group. Training started at 1 week after SCI. At this point, "soft tissue had healed sufficiently, and occasional appearance of red porphyrin expression around the eyes, a symptom associated with stress, disappeared within a week post-SCI." A manual body weight support was provided by the trainers. Jayaraman et al. (2012) explained that "muscle regenerative response was initiated only in the slow-twitch soleus muscle in the initial 2 weeks following SCI, the addition of locomotor training of 1 week led to a significant increase in soleus regenerative process. No significant regenerative process was observed in the fast-twitch tibialis anterior muscle." The results by Stevens et al. (2006) were as follows: "locomotor training resulted in a significant improvement in the overall locomotor function (32% improvement in BBB scores, 38% greater peak soleus tetanic forces, a 9 % decrease in muscle fatigue, 23% larger muscle fiber CSA (cross sectional area). There is a good correlation between the BBB scores of injured animals and the peak soleus muscle force." Liu et al. (2010) reported that "treadmill training triggered increased expression of mRNA of IGF1 (insulin growth factor 1) and IGFBP4 (IGF-binding protein), and concurrent reduction of IGFBP5 mRNA in soleus muscle. There was an increase in expression of muscle regeneration markers in soleus muscle: embryonic myosin and Pax7 positive nuclei in small muscle fibers, and MRFs (myogenic regulatory factors), myogenin and MyoD."

Robotic training

The experimental techniques for treadmill training with partial body weight support have many variations. It is rather difficult in experimental studies to support the animal's body in a steady state during the locomotor training. To resolve this problem, robotic training has been studied, in which rats were supported in a harness and aided in walking by robotic arms fixed at their hind limbs.

Nessler et al. (2006) showed that weight-supported steps are important for the locomotor recovery. They assessed the ability of a robotic device ("rat stepper") to reduce the locomotor impairment following a contusion injury in rats (Fig. 6). Several measures improved significantly during their 4-week training: swing velocity, step height, step length, hind limb coordination, and the ability to support body weight. They proposed that "it is the quality of weight-supported steps, rather than the quantity, that best reflects locomotor recovery after contusion injury." On the other hand, Cha et al. (2007) examined on the locomotor improvement of the frequency of stepping. Rats were trained on a treadmill: "one group received 1,000 steps/training session, and the other group received 100 steps/training session. During training, the robotic device imposed the maximum amount of weight that each rat could bear on the hind limbs. After 4 weeks of

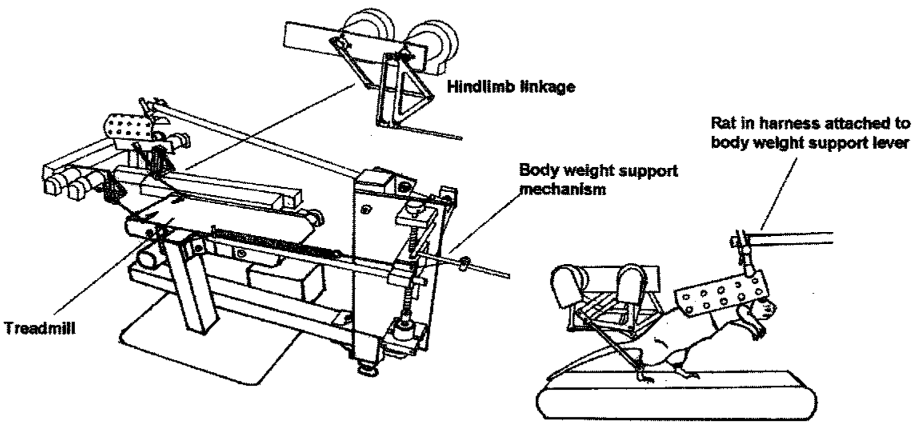

Fig. 6 Prototype version of the "rat stepper." The rat is placed in a cloth harness and attached to the end of the body weight support (BWS) lever. The orientation of the rat's torso is adjustable, and the amount of weight support delivered to the animal is precisely controlled. The rat steps bipedally in the device, and the small robotic arms attach to each hind limb with neoprence straps. These robotic arms can impart forces to the animal, or simply record the trajectories of each ankle, similar to optical motion capture. (Nessler et al. 2006, with permission)

training, the quality of stepping in the group that received 1,000 steps/training session much improved, while little improvement in the quality of stepping was observed in the group that received only 100 steps/training session. This indicates that the ability of the lumbar spinal cord to improve the locomotor activity is dependent on the number of repetitions during treadmill training."

In the robotic training, rats moved their hind limbs without weight bearing on the foot sole. Therefore, there may have been almost no effective sensory input from the hind limbs. In addition, this robotic walking did not take a four-footed pattern, which meant that the hip joints were not fully extended, the center of gravity of their body was situated at a higher level than normal, and coordinated fore-limb movement was not achieved. Lee et al. (2011) examined whether assisted-as-needed (AAN) robotic training was better than the fully assisted (FA) training in rats with incomplete SCI. They showed that the locomotor recovery was greater in the AAN (partial body weight-supported) robotic training than the fully assisted training. "FA training drove the ankle along the desired trajectory, whereas greater variety in ankle movements occurred during AAN training. After 4-week training, locomotor recovery was greater in the AAN group, as demonstrated by the ability to generate steps without assistance, more normal-like kinematic characteristics, and greater EMG activity." They conclude that the flexible robotic assistance might facilitate learning to step after a SCI. This would mean that inputs from the outside are important for promoting the locomotor circuitry in the lesion of the spinal cord.

Clinical application

Clinically, the gait orthosis is used, in combination with treadmill, to maintain posture and/or to support the body weight of patients. Dietz et al. (2002) carried out the walking training with the driven gait orthosis "Lokomat" on a treadmill. Patients performed four different kinds of walking: "normal walking, hip walking, unilateral walking, and air stepping. The hip walking involves the bilateral pattern of leg muscle activation while walking with blocked knee movements." First three types of walking were performed with a partial load of 30% of body weight. EMG was recorded in the rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG) muscles. Subjects were 6 patients with a chronic complete para/tetraplegia, and 3 healthy persons. The treadmill speed was 1.9 km/h. The unilateral walking allowed "only the right leg to perform stepping movements, while the movements of the whole left leg were blocked. No visible EMG activity was seen in the left leg of the patients, while, the healthy subjects showed the pattern of leg muscle activation in the left leg." The air stepping condition was walking with no body weight load (complete body weight-bearing). In this condition, there was no muscle activity in the legs. They concluded that "the afferent input from hip joints in combination with that from load receptors, plays a crucial role in the generation of locomotor activity in the isolated human spinal cord."

Colombo et al. (2001) report a case study of the effects of manually assisted locomotor training in paraplegic patients as compared to an automated training by a driven gait orthosis. They assessed two patients, one with an incomplete lesion at C3, and the second with a complete lesion at C5. The EMG activity was recorded from the leg muscles RF, BF, MG, and TA. Results were as follows: "there was no significant difference between the two training methods according to the leg muscle EMG. It was concluded that neuronal centers in the spinal cord become activated in a similar way by the manually assisted and the automated locomotor training." The importance of the neuronal center was emphasized by Kojima et al. (1998). They reported on rehabilitation therapy using orthotic gait in patients with complete SCI. EMG was recorded from RF, BF, MG, soleus, and TA. "EMG showed some similarities to that of the infant stepping or immature gait." This suggests the existence of a motor mechanism, i.e. the CPG that originates in the spinal cord.

Hesse et al. (1999) reported beneficial effects of treadmill training in patients with a harness. Patients were harness-secured by a full body weight bearing (unsupported), or by a partial loading of 15% and 30% of the body weight. Treadmill speed was 0.27 m/sec with a range of 0.1 m/sec to 0.35 m/sec. In contrast, control patients walked on the floor, a 15-m walkway, with a mean walking velocity of 0.32 m/sec with a range of 0.18 m/sec to 0.48 m/sec. Gait analysis was performed using parameters as follows: velocity, cadence, cycle stance, swing, double support duration, vertical ground reaction forces at heel-on and toe-off, stride length, and symmetry ratios for stance and swing. EMG was recorded from TA, MG, BF, vastus lateralis, MG, and elector spinae (Fig. 7). Their conclusion was that "treadmill training with partial body weight support in hemiparetic subjects allowed them to practice a favorable gait characterized by a greater stimulus for balance training because of the prolonged single stance period of the affected limb, a higher symmetry, less plantar flexor spasticity, and a more regular activation pattern of the shank muscle as compared with floor walking."

In randomized clinical trials using body weight support method, Dobkin et al. (2003) performed body-weight supported treadmill training (BWSTT) of patients with SCI. "The therapists provided verbal and tactile cues to facilitate kinematic, kinetic, and temporal features of walking. Subjects were randomly assigned to a conventional therapy program for mobility versus the same intensity and duration of a combination of BWSTT and over-ground locomotor retraining. One hundred and forty-six subjects were entered for 12 weeks of intervention. The trial methodology offers a model for the feasibility of translating neuroscientific experiments into a randomized clinical trial to develop evidence-based rehabilitation practices."

Sensory inputs are also important in clinical locomotor training. Harkema et al. (1997) examined the role of sensory input for modulating the efferent motor pattern in humans. "This study examined the role of sensory information related to lower extremity weight bearing in modulating the efferent motor patterns of spinal cord injured subjects. The level of loading, EMG patterns, and kinematics of the lower limbs were studied manually assisted or unassisted stepping on a treadmill with body weight support. The relationship among lumbosacral motor pool activity (soleus, MG, and TA), limb load, muscle-tendon length, and velocity of muscle tendon length change were examined. The data suggest that the level of loading on the lower limbs provides cues that enable the human lumbosacral spinal cord to modulate efferent output in a manner that may facilitate the generation of stepping."

Others

A three dimensional (3D) recording of locomotor behavior was reported by Couto et al. (2008). They used a 3D technique to determine hind limb kinematics during treadmill locomotion. "A 2D method cannot record the external or internal rotations of the foot because this movement occurs in the transverse plane. The maximal precision and accuracy of the kinematic values are expected when the experimental protocol includes a 3D motion analysis methodology." They presented the following data: walking in normal rats, treadmill speed: 40 cm/s, step cycle duration: 337.1±39.0 ms, stance duration: 231.7±31.4 ms, swing duration: 105.7±17.0 ms, and stride length: 13.6±1.3 cm, and walking in rats with SCI 2 weeks post-operation, treadmill speed: 30 cm/s, step cycle duration: 423.7±47.9 ms, stance duration: 314.0±49.2 ms, swing duration: 110.0±15.5 ms, and stride length: 12.8±1.1 cm.

Melanie et al. (2006) used a wide variety of parameters for assessing locomotor behaviors. They examined improvement of locomotor behaviors after SCI, without treadmill training. They

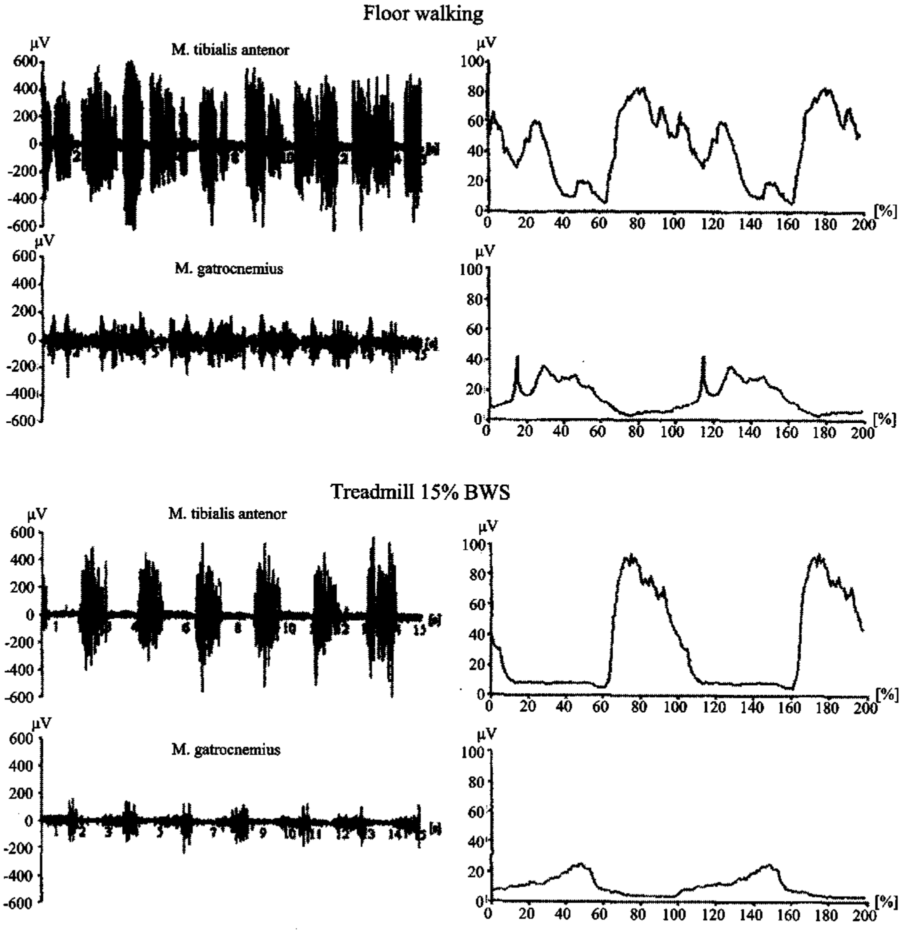

Fig. 7 Raw and averaged data for the gait cycle-normalized activity of the left tibialis anterior and gastrocnemius muscles of a left hemiparetic subject walking on the floor and on the treadmill with 15% body weight support. (Hesse et al. 1999, with permission)

used the Ohio State University Device as described above for SCI. No locomotor training was employed. "BBB ratings improved during recovery. The number of hind limb foot-faults on the horizontal ladder increased after injury and remained elevated during the recovery period. Some locomotor parameters (velocity, stride length, stride duration, stance duration) of the injured rats improved slightly, some (interlimb coordination, swing duration, forelimb base of support, hind paw angle) did not change, and others (hind limb base of support) declined, during the recovery period. These results show that gross locomotor skill improves after SCI, while recovery of fine locomotor function was more limited."

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