Combined Effects of Acrobatic Exercise and Magnetic Stimulation on the Functional Recovery after Spinal Cord Lesions

Zaghloul Ahmed^{1,2} and Andrzej Wieraszko²

Abstract

The objective of the study was to determine whether physical exercise combined with epidural spinal cord magnetic stimulation could improve recovery after injury of the spinal cord. Spinal cord lesioning in mice resulted in reduced locomotor function and negatively affected the muscle strength tested *in vitro*. Acrobatic exercise attenuated the behavioral effects of spinal cord injury. The exposure to magnetic fields facilitated further this improvement. The progress in behavioral recovery was correlated with reduced muscle degeneration and enhanced muscle contraction. The acrobatic exercise combined with stimulation with magnetic fields significantly facilitates behavioral recovery and muscle physiology in mice following spinal cord injury.

Key words: acrobatic exercise; injury; magnetic stimulation; recovery; spinal cord

Introduction

SPINAL CORD INJURY (SCI) occurs throughout the world with an annual incidence of 15–40 cases per million (www.rick-hansenregistry.org/page192.htm). Although the rate of survival and quality of care has improved over the years, the progress in improving functional recovery has occurred at a much slower pace. Current progress on research into spinal cord recovery after injury was discussed at the Symposium during the 2007 Society for Neuroscience meeting and has been summarized by Rossignol et al. (2007). It is apparent that, since the dogma that spinal cord neurons cannot regenerate axons has been abolished (Richardson et al., 1984), more research has focused on enhancement of the natural ability of the spinal cord to induce neuroplasticity and overcome damage (Rossignol et al., 2007). Current methods of intervention (chemical, surgical, rehabilitation) provide modest benefits and, as indicated by Baptiste and Fehlings (2007), “there is a critical need to identify novel approaches to treat, or repair the injured spinal cord.”

Lesions to the spinal cord are rarely complete. Typically, there is a bundle of axons that survive the impact and could become a target of treatment aimed at functional recovery.

Indeed, behavioral (Rossignol et al., 2007) and electrical (Brus-Ramer et al., 2007) activation of these axons improves locomotion and connectivity within the injured spinal cord. It has also been demonstrated that exercise improves functional recovery after SCI (Hutchinson et al., 2004; Devillard et al., 2007; Thuret et al., 2006). Motor skills training (e.g., acrobatic tasks) has an even greater effect on structural plasticity (number and size of synapses) and functional recovery after brain damage than repetitive exercises (e.g., running) (Jones et al., 1999). However, even the benefits of acrobatic exercise are still relatively modest. Therefore, we decided to combine skills training with magnetic stimulation (MS). This initiative was based on our previous results, which showed that exposure of the brain *in vivo* to repetitive transcranial magnetic stimulation (rTMS) modulates animal behavior and excitability of the central (Ahmed and Wieraszko, 2006a) and peripheral (Ahmed and Wieraszko, 2006b) nervous systems.

Stimulation of the nervous tissue by rTMS occurs according to Faraday’s law of electromagnetic induction and follows principles already extensively discussed (Barker, 2002; Ruohonen and Ilmoniemi, 2002; Bohning 2000; Nahas et al., 2001; Post and Keck, 2001). The rTMS apparatus generates a

Departments of ¹Physical Therapy and ²Biology, and CSI/IBR Center for Developmental Neuroscience, The College of Staten Island/CUNY, Staten Island, New York.

short pulse of an electrical current, which produces a short-lasting magnetic pulse of high intensity (up to 4 Tesla [T]) in the magnetic electrode. The changing magnetic field induces electrical, stimulating current in the tissue located in the proximity of the electrode. Thus, the tissue is stimulated by the electrical current transferred from the generating device to the brain via magnetic fields. There are several recently published reviews and books describing application of rTMS in several disorders of the central nervous system (CNS) (Pascual-Leone, et al., 2002; Walsh and Pascual-Leone, 2003), although reports describing the application of this type of stimulation directly to the site of the spinal cord/nerve injury are rare (Wilson and Jagadeesh, 1976; Orgel et al., 1984). Cranial application of rTMS was reported to significantly improve locomotor recovery after spinal cord lesions in rats (Poirrier et al., 2004) and humans (Belci et al., 2004). The mechanism by which rehabilitation and physical therapy improves function, after SCI, is still not well understood (Thuret et al., 2006), and there are no reports about the influence of skilled motor training, combined with MS, on functional recovery after SCI.

We undertook our investigation to test the hypothesis that the exposure of damaged tissue of the spinal cord to rTMS (hereafter called in our paper "magnetic stimulation," or "MS") would prime this tissue to respond more favorably to the acrobatic exercise. Although the results are preliminary, we consider them highly promising and appropriate for further, more detailed investigations.

Methods

Exposure to magnetic fields

Normally, rTMS involves MS of the brain through the bones of the skull. However, since in our experiments we did not stimulate the brain, the term "transcranial" is inaccurate, and we prefer the term "magnetic stimulation" (MS). MS was delivered with a circular coil (5 cm in diameter) connected to MagStim Rapid (MagStim, UK). Since the mouse body is relatively small as compared to the magnetic coil, we used a muscle preparation to determine the range and the strength of the electromagnetic magnetic field. The animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg), the skin covering the hind limb was removed, and the distal end of the gastrocnemius muscle was dissected. The entire muscle was then freed from the surrounding tissue. The proximal attachment was left intact. The gastrocnemius tendon was threaded with surgical silk and attached to the myograph. The sciatic nerve was exposed for about 3 cm and freed from the tissue underneath. A piece of insulating plastic was placed between the nerve and underlying tissue. It was determined that the nerve was completely insulated electrically from the tissue. While the animal was rigidly fixed to the base, the hind limbs were fixed at many points to ensure that no movement could be transmitted from the animal's body to the myograph through the gastrocnemius muscle. The magnetic coil, activated every 5 sec, was slowly moved toward the muscle. The muscle contracted if the magnetic coil was less than 1 cm from the muscle. When the coil was placed in the proximity of the sciatic nerve, which innervates gastrocnemius muscle, it contracted with greater amplitude, indicating that the action potential induced by MS in the

nerve contributed much more to the twitch of the muscle than direct MS of the muscles itself. The muscle contracted also when the magnetic coil was placed directly above the skull. When the coil was located above the spinal cord, 3 cm caudally from the brain, even stronger twitches were observed (Fig. 1B). However, the muscle did not contract if the magnetic coil stimulated the brain of the animals with spinal cord transection.

Following these control experiments, MS was applied to experimental animals. Each animal received 450 pulses of 1.5 T delivered at the frequency of 1 Hz. The duration of each pulse was 450 μ sec, and the rising time was 60 μ sec. Stimulation with MagStim lasted 7.5 min and was applied once a day, consecutively for 24 days. During exposure, animals were restrained in a custom-made wooden box (Fig. 2B). A hole in the wall of the box allowed us to pass the tail outside and to fix it with Velcro tape to the side of the box. Two rubber strips were used to restrict the mouse's body in the downward direction. The coil, 5 cm in diameter, was placed on the top of the box a few millimeters from the spine. The forelimbs, the head, and the cervical region were not covered by the coil. This restraining box was found to be very effective in holding the mice motionless and reasonably comfortable during the treatment.

Exercises

Acrobatic exercises were employed according to Jones et al. (1999). Animals were required to complete five activities: (1) to traverse five ropes of the same length (50 cm) but different diameters (1, 2, 2.5, 3, and 3.5 cm); animals were assisted to balance themselves on the ropes; (2) to traverse a ladder with 2.5-cm spaced rungs; (3) to traverse a rod with 1.5-cm diameter and 50-cm length; (4) to walk on a grid platform (50 $\times$ 10 cm, with 2.5-cm² openings); and (5) to walk through a barrier course (5–10 cm high; Fig. 2C). It took 60 min for individual animals to complete all five tasks. Normal control (NC) animals were not subjected to the exercise protocol, since it did not change their behavior.

Surgical procedure

Thirteen CD-1, 35-day-old female mice (24–30 g) were used. Following 3 days of acclimatization to exercise and the open-field environment, animals were deeply anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg), and op-

FIG. 1. Magnitude of the muscle twitches induced by magnetic stimulation (MS). (A) Muscle twitch induced by the magnetic coil placed just above the head. (B) Muscle twitch induced by magnetic coil placed above the spinal cord.

FIG. 2. Lesion verification and experimental procedures. (A) Coronal section of the spinal cord depicting averaged area of the spinal cord lesion. Extent of damage has been evaluated as described in the Methods (see also Fig. 9). (B) Position of mouse in restraining wooden box and the location of the magnetic coil. (C) Examples of the acrobatic exercise.

erated. The T13 of the spinal cord was exposed by T10 laminectomy, and the lesion was made with micro-scissors. Micro-scissors with 0.5-mm blades were lowered to cut the spinal cord on both sides except the most anterior portions (Fig. 2A). The procedure was performed exactly the same way for all animals. Two days after laminectomy and incomplete spinal cord transection, 10 injured animals were randomly divided into five, differently treated groups: (1) three animals received spinal MS followed by acrobatic exercises (EX+MS); (2) two animals exercised, but were not exposed to magnetic stimulation (EX); (3) two animals were exposed to magnetic stimulation, but did not exercise (MS); (4) three animals served as injured control (InC), with neither acrobatic exercise nor MS; and (5) three animals were neither operated, nor treated in any way and served as normal controls (NC). MS and acrobatic exercises were administered daily for 24 days starting 2 days after SCI.

Behavioral assessment

Behavioral tests were conducted once a week, and the animals were evaluated according to the Basso Mouse Scale (BMS) and horizontal-ladder test. The BMS for locomotion was used to determine the functional outcomes of the different treatments (Basso et al., 2006). The mice show a stereotyped sequence of locomotor recovery after SCI. Joints movements were followed by weight support, stepping, fore-hind limb coordination, normalizing foot placement, and tail positioning. The range for the BMS score for mice is 0–9, where 0 is when a mouse has no movement and 9 is when movements are perfectly normal. Mice were placed in a plastic tub (75 × 50 cm), and BMS scores were assigned by two observers following a 4-min test. The observers scored each mouse individually and then assigned the consensus score. Data were collected from each hindlimb (left and right) separately, and then averaged for comparison.

The horizontal-ladder test evaluates the quality of movement during walking in difficult and challenging conditions. The animals walked 20 steps (counted for each hind limb) across a horizontal-ladder with constant rung spacing (2.5 × 2.5 cm). Evaluation was performed by two investigators. While

one of them observed and counted the steps on the left hind limb, the other evaluated in the same way the performance of the right limb. The error ratio was calculated as the number of missteps divided by the total number of steps (20 steps). A misstep occurs when the hind paw is not placed on rings, but dangles downward in the ladder holes instead. All animals were marked, and their identity was not known to the testers.

Evaluation of muscle contraction

Isometric muscle contraction reflects the status of the muscle physiology. Following SCI, muscles of involved limbs undergo several changes that include spasticity, weakness, faster fatigue, contractile change, motor unit reduction, and decrease in the firing rate of motor units (Shah et al., 2006). Those changes are mutually interconnected, and evaluation of isometric muscle contraction has been found to correlate strongly with modification of the muscle weakness, strength (Shah et al., 2006), and improved walking (Kim et al., 2004). Therefore, using myograph, we evaluated properties of the gastrocnemius muscles in different groups of animals. The gastrocnemius muscles of both hind limbs were dissected out. One thread was tied to the dissected calcaneus tendon. The lower end of the femur bone, above the femoral condyles and at the knee joint, was cut to preserve the attachment of the gastrocnemius and keep the whole muscle intact. Another thread was attached to the bone stump. The muscles were then incubated at 33°C in oxygenated (95%:5% O₂:CO₂) Tyrod's solution containing (mM): NaCl 125, KCl 5, MgCl₂ 0.5, Na₂HPO₄ 0.4, CaCl₂ 1.8, NaHCO₃ 25, glucose 5.5. Muscles were individually mounted on refined myograph (Kent Scientific Corp. USA), and the muscle length was adjusted for maximum twitch force (optimal length). Following a 5-min adaptation period, three parameters for each muscle were tested:

1. The relation between the stimulus intensity and the force generated by individual twitches was measured. Muscles were stimulated with stimuli of constant duration (0.2 msec) and with intensity increasing in 10-V intervals from 10 to 100 V.

2. The relation between the frequency of stimulation and the force generated by the muscle during tetanic contraction was measured. Tetanus was induced by stimuli (intensity 60 V, duration 0.2 msec) applied for 3 sec with the frequency rising in 10-Hz intervals from 10 to 100 Hz. The force-frequency relationship and the force of contraction were evaluated from the graphs of the pen recorder. To prevent potentiation, the muscle rested 2 min between each tetanus, and 20 min before the next test.
3. The fatigability of the muscle was tested by application of 20 1-sec stimuli with frequency of 40 Hz and intensity of 60 V repeated 20 times. Following testing, muscles were trimmed from connective tissue and bone, and weighed, frozen, and taken for sectioning.

Staining of the muscles

Each frozen muscle was sliced with the cryostat, and eight transverse sections 20 μm thick, collected every 300 μm , were mounted on gelatin-coated slides. Sections were then stained with hematoxylin and eosin (H&E) following standard procedure. Slices were immersed in Harris hematoxylin for 3 min, rinsed in tap water, and then left under slowly dripping water for approximately 20 min until the blue color was sharpened. Next, the slices were immersed in eosin stain for 1–2 min, rinsed in tap water, and dehydrated by transferring in turn to several alcohol solutions of increasing concentrations (50%, 70%, 95%, and 100%). Following clearing with xylene, the sections were coverslipped and photographed with a Zeiss Axiophot microscopy camera. Digitized photographs were exported to Photoshop (CS3 Extended) and analyzed. To investigate changes in muscle morphology, the cross-sectional area of individual muscle fibers and the void area of muscle tissue was evaluated.

Staining of the spinal cord

The whole vertebral column was isolated and placed for 24 h in 4% paraformaldehyde in phosphate-buffered saline (PBS; 7.4 pH), and a 1-cm-long segment including the lesion epicenter or laminectomy site was removed. The lesion epicenter and 2 mm rostral and 2 mm caudal were sliced in a coronal plane at -20°C . One slide containing eight slices from each rostral, caudal, and lesion epicenter regions was stained with luxol fast blue. The lesion epicenter was identified as the least myelin-stained section. The slices were photographed and exported to Photoshop (CS3 Extended). Percentage of spared or regenerated white matter was calculated from pixels of white matter area, divided by pixels of total cross-sectional area of the cord measured at the lesion epicenter.

Results

Magnetic stimulation

Since the mouse body is relatively small compared to the magnetic coil, we used muscle preparation *in vivo* to evaluate the range and strength of the electromagnetic field reaching the spinal cord. It has been determined that, when properly applied, MS was focused on the spinal cord and sciatic nerve, initiating contraction of the gastrocnemius muscle. Twitches of the muscle were induced also when the coil was located either above the skull (Fig. 1A) or above the spinal cord (Fig. 1B). However, in the animals with transected

spinal cord, the twitch of the muscle occurred only when the coil was located below the transection. These results indicate that, while there was some direct MS of the muscle, the major stimulatory impulse to induce muscle twitch originated in exposed spinal cord and/or sciatic nerve. The location of the coil and narrow range of the magnetic field excluded the possibility that the action potential, initiating the twitch of the muscle, originated in the brain. The group of the animals used to evaluate MS was not included in any other experiments.

Behavioral tests

BMS evaluates general ability of the animals to move. As shown in Figure 3A, acrobatic exercise applied together with the exposure to magnetic fields was the most effective in stimulating improvement. Since this combined treatment gave the best results, we evaluated the relation between duration of the postoperative period and the level of recovery using the BMS. As illustrated in Figure 3B, InC did not show any improvement in walking over the 24-day period. In contrast, animals that were exercised and exposed to MS reached almost 7 on the scale. Although the improvement occurred gradually, reaching the highest value at day 24, the difference between controls and exercising/MS-exposed animals became statistically significant at day 10 (repeated-measures analysis of variance [ANOVA] followed by post hoc test, $p < 0.05$). Post hoc analysis revealed that the recovery was accelerated by the end of our testing period, since there was a statistically significant difference between day 17 and 24 in the performance of the exercising/MS-treated animals. One-way ANOVA, followed with post hoc results, showed significantly higher improvement in BMS scores in EX+MS animals than in all of the other groups. Although the exercising (EX) and MS-exposed animals (MS) showed similar improvement, due to the lower variability of the data, only the MS group performed significantly better ($p < 0.05$; Fig. 3A) than controls (InC) animals.

The horizontal-ladder test evaluated the ability of the animals to place limbs correctly under stressful and challenging conditions. While the injured, non-treated animals (InC) showed the highest incidents of missteps (InC animals; Fig. 4), the animals that received MS and acrobatic exercises performed much better (EX+MS; Fig. 4). Although the performance of InC, EX, and MS groups was statistically different ($p < 0.05$) from control group (NC), there was no statistically significant difference between controls (NC) and exercising animals exposed to MS (one-way ANOVA followed by post hoc analysis).

Muscle contraction in vitro

Properties of the gastrocnemius muscle were tested *in vitro* by evaluating the force of the muscle contraction, response to tetanic stimulation and the resistance to fatigue. As shown in Figure 5A, the strongest single twitch contraction force was expressed by the muscle obtained from exercising animals (EX). Simultaneously, the muscles from these animals expressed the longest twitch-to-peak time (Fig. 5B), and half-relaxation time (Fig. 5C). These two parameters express the time which is required to reach the maximum contraction following stimulation, and half of the time needed by muscle to relax after reaching the peak, respectively. All these properties of the twitch contraction, which were significantly

FIG. 3. Influence of different types of treatment on the mouse locomotor recovery after spinal cord injury (SCI). **(A)** Change in the Basso Mouse Scale (BMS) observed in different groups of animals 24 days after injury. Note the remarkable recovery of the animals subjected to both treatments: exercise and magnetic stimulation (EX+MS). **(B)** Progress in the recovery of InC and exercising, MS-exposed animals (EX+MS), evaluated with the Basso scale over the period of 24 days. BMS scores were standardized by calculating a percentage of change of normal score (or 9) as follows: d2 score minus d24 score, divided by 9, and multiplied by 100. This allowed for comparison between groups regardless of their baseline scores. Repeated-measures analysis of variance (ANOVA), followed by post hoc test; X indicates $p < 0.05$. InC, injured controls; EX, exercise alone; MS, MS alone; EX+MS, exercise combined with the exposure to MS.

enhanced in exercising animals, represent spastic syndrome characteristics. The injured, non-treated controls (InC) generated the weakest response to stimulation. Three other groups demonstrated the force of the muscle contraction to

be slightly higher than InC, but much lower than exercising animals. When the force of the muscle contraction was normalized by taking into account muscle weight the results were identical.

Examples of the response of gastrocnemius muscle from different groups of the animals to tetanic stimulation are shown in Figure 6A. The forces of the muscle contraction generated by gastrocnemius muscle from control and exercising/MS-treated animals were the highest and almost equal to each other. While the weakest force was generated by the muscles from InC, the muscles from exercising (EX) and MS-exposed animals demonstrated similar, intermediate values (Fig. 6A). Averaged results obtained from six muscles are shown in Figure 6B. Although the reduction in the tetanic force expressed by the muscle from exercising animals (EX) was lower than from MS-exposed animals, the results were not statistically significant due to their high variability.

Induction of the fused tetanus was tested by stimulating the muscles with different frequencies. As depicted in Figure 7A, while the muscles from exercising animals (EX) and exercising/exposed (EX+MS) fused already at 40 Hz, the muscles from two other groups of treated animals (InC and MS) fused at much higher frequencies of stimulation (60–80 Hz). Muscles from the control, untreated animals fused at the highest frequency of stimulation of 90 Hz (Fig. 7A). Figure 7B shows averaged tetanic force normalized for the mass of the muscle. Muscles from InC generated the lowest normalized tetanic force, which may indicate replacement of atrophied muscle tissues by connective tissues. That is consistent with our finding of increased void areas between muscle fibers in muscles from InC (Fig. 11C).

FIG. 4. Performance of different groups of the animals in the horizontal-ladder test. The performance was expressed as a number of missteps of hind limbs. In confirmation of the results with Basso Mouse Scale (BMS), the animals that exercised and were exposed to magnetic stimulation (MS) showed the best performance. Although the horizontal-ladder test was performed four times, the figure illustrates the final evaluation only. X indicates results statistically different from the noninjured controls (X indicates $p < 0.05$, one-way analysis of variance [ANOVA] followed by post hoc analysis). Abbreviations as in legend to Figure 3.

In support of the above data, we determined that the half-fall time of the tetanic contraction, defined as the time needed for the maximal tetanic force to be reduced by half after stimulation, was significantly extended in exercising (EX) animals. While in all groups of animals, the half-fall time ranged from 42 to 69 msec, in exercising animals it was much longer, reaching over 160 msec (162.5 ± 21.7 , $p < 0.05$, one-way ANOVA followed by post hoc Tukey test). This result correlates with symptoms of spasticity, which were expressed in the strongest way in EX animals.

Fatigue of muscles, expressed as a decrease in tetanic force in response to repeated stimulation, is depicted in Figure 8A. Interestingly, in all groups except InC, the tetanus force increased soon after initiation of stimulation. The increase was most pronounced in exercising/exposed animals (EX+MS) and exceeded the enhancement observed in the muscles from control animals (NC). The increase in exercising (EX) and MS-exposed (MS) groups of animals was similar. In all groups, the increase was short-lasting and was followed by a gradual decrease of the tetanic force while the stimulation continued. Muscles from InC responded very weakly to the stimulation, and their force of contraction rapidly declined to almost zero. To characterize the fatigability of the muscles, the tetanic force of the last tetanus was divided by the force of the first one to calculate the fatigue index (Fig. 8B). The low value of the index indicates high fatigability of the muscle. While the InC and exercising animals (EX) have the lowest and similar fatigue indexes, the muscles from exercising/exposed (EX+MS) and control animals express equal fatigue indexes. This implies that acrobatic exercise combined with MS either prevents muscle deterioration or facilitates its recovery.

Lesions

The histological verification of lesions revealed that the total area of lesion epicenter and the total spared area were very similar in all animals (Fig. 9A). However, the area occupied by spared axons differed significantly (Fig. 9B). While the axon area was only $29.33 \pm 0.88\%$ in InC, it was $40.66 \pm 1.20\%$ and $38.00 \pm 1.15\%$ in EX+MS and EX groups, respectively. The results, shown in Figure 9B, suggest that MS may increase corticospinal tracts or local interneurons sprouting into the lesion epicenter region. This is consistent with the most recent findings that electrical stimulation of corticospinal tracts, which resembles MS, enhanced sprouting into the lesioned spinal cord region (Brus-Ramer et al., 2007).

FIG. 5. Properties of individual twitches in different groups of the muscles. **(A)** The maximum force of a single twitch generated by the muscles stimulated with increasing stimulus strength. Note that the twitch force recorded from exercising animals (EX) is much higher than in all other groups. **(B)** Twitch peak time, a time required by the muscle to achieve maximal contraction following stimulation (X indicates $p < 0.5$). **(C)** Half-relaxation time, a time during which the muscle relaxes to half of its maximal force (X indicates $p < 0.5$). One-way analysis of variance (ANOVA) followed by post hoc was used to evaluate the data in B and C. Abbreviations as in legend to Figure 3.

FIG. 6. Force generated by the muscles exposed to tetanic stimulation. (A) Representative contractions recorded from the muscles obtained from different groups of the animals. Note that, while injured controls (InC) contract very weakly, the muscles from exercising/magnetic stimulation (MS) exposed animals contract almost like normal controls (NC). (B) Averaged results illustrating the muscle force generated in response to tetanic stimulation. While X represents statistically significant difference ($p < 0.05$) between controls (C) and InC, XX indicates that the results obtained from InC and exercising/MS exposed are statistically different ($p < 0.05$) from InC.

Muscle morphology

Weight of the wet muscle evaluated after dissection was the lowest in InC and in the animals exposed to MS (Table 1). Exercising animals (EX) and the exercising animals exposed to magnetic fields (EX+MS) had similar muscle weight, which was approximately 20% lower than in control animals. However, the muscle to body ratio decreased in InC, exercising (EX), and magnetic field exposed (MS) animals, although this decrease was significant for InC and MS animals only ($p < 0.05$, one-way ANOVA followed by post hoc analysis). Exercising animals (EX) and exercising and exposed animals (EX+MS) demonstrated muscle to body ratio very similar to controls and significantly higher than InC (Table 1).

In agreement with the observed changes in the muscle weight, the cross-sectional area of the muscle was reduced (to approximately 30% of control) in InC. While the muscles from exercising (EX) and magnetic field exposed (MS) animals had the area reduced approximately by half, the muscles from exercising/exposed (animals EX+MS) were prac-

tically not changed. Simultaneously, while the void area (i.e., not occupied by the muscle tissue) increased dramatically in InC, it was almost unchanged in EX+MS animals (Figs. 10 and 11C). The animals in EX and MS groups showed intermediate values. These observations correlate well with the size of the muscle fibers. While the fibers in InC were the smallest and their size varied significantly, the exercising animals exposed to magnetic field showed larger muscle fibers and much lower variability (Fig. 11A,B). The void area was

B

FIG. 7. The influence of frequency of stimulation on maximum tetanic force. (A) Increasing frequency of stimulation enhanced the maximum tetanic force until the muscle reached the fused tetanus expressed as plateau. Note that exercising (EX) and exercising/magnetic stimulation exposed (EX+MS) animals reached plateau at 40 Hz, while in other groups it was shifted to the right to reach the the maximum at 60 and 90 Hz for injured controls (InC) and noninjured controls (NC), respectively. The muscles from magnetic exposed animals (MS) reached the plateau at 80 Hz. (B) Expression of the average maximum tetanic force, normalized considering the muscle weight. X and XX indicate data statistically different ($p < 0.05$) as compared to NC and InC, respectively. Other abbreviations as in legend to Figure 3.

FIG. 8. Fatigue of the muscles induced by high-frequency stimulation. (A) Change in the tetanic force induced by 40-Hz/60-V stimuli repeated once per second for 20 sec. The response of the muscles to each of 20 stimuli is plotted against muscle force. Arrow indicates the first tetanus. Note that tetanic force was initially potentiated to quickly decline at the end of the stimulation. (B) Fatigue index. Magnitude of the last tetanic force was divided by the first one to calculate the fatigue index; X indicates $p < 0.05$, one-way analysis of variance (ANOVA) followed with post hoc or Kruskal-Wallis Test.

inversely correlated with the size of the muscle fibers. InC had dramatically larger void area, as compared with all other groups. Evidently, the acrobatic exercise combined with magnetic exposure reduced decline in the muscle fiber size and the atrophy of the muscle fibers.

Discussion

We presented a novel approach to attenuate the effects of spinal cord lesions by combining acrobatic exercise with MS. Since the stimulating coil was larger than the animal, not only the spinal cord but several other structures (including peripheral nerves and muscles) were stimulated as well. However, the animals which received MS only (MS group) performed much worse, than the animals which received combined treatment. Therefore, although the beneficial effects of peripheral stimulation were reported (Krause et al.,

2004), it is very unlikely that its contribution to our results was significant.

It has been established in the past (Goldshmit et al., 2008; Fischer et al., 2007; Dobkin et al 2007; Barbeau et al., 2006), and confirmed by us, that different types of physical exercise improved animal's motor recovery. We have demonstrated for the first time that MS combined with these acrobatic exercises improves this recovery even further. The influence of magnetic fields on the nervous tissue remains a controversial topic (Wassermann and Lisanby, 2001). Although several different types of MSs have been used for research and clinical applications (Wassermann and Lisanby, 2001), we have selected stimulation with magnetic fields used by us in the past (Ahmed and Wieraszko, 2006a) which are generated by FDA approved equipment. These strong magnetic fields easily penetrate the tissue and create electrical current in neurons, which in response generate action potentials. It should be emphasized that, according to our procedure the animals were stimulated electrically (via magnetic fields) and by magnetic fields themselves.

The beneficial action of different types of MS (Sisken et al., 1989; 1990, Crowe, 2003; Nichols et al., 2004), including rTMS (George and Belmaker, 1997; Post and Keck 2001; Pascual-

FIG. 9. Lesion area. (A) Area of the lesioned site was very similar in all groups of animals. The area was calculated from the computer images obtained from the sections of the spinal cord. (B) Axon area observed within a lesion site. While the area was the smallest in injured controls (InC), all other groups of the animals demonstrated the axon area which was significantly greater, and smaller as compared to InC and non-injured controls, respectively; X indicates $p < 0.05$.

TABLE 1. MUSCLE-TO-BODY RATIO IN NORMAL AND INJURED ANIMALS

Group	Number of muscles	Mice weight (g)	Wet muscle weight (mg)	Muscle-to-body weight ratio, (mg/g)
Normal control	8	35.3 $\pm$ 16.2	320.0 $\pm$ 94.4	8.80 $\pm$ 1.5
Injured control	6	24.5 $\pm$ 0.7	130.5 $\pm$ 42.5*	5.42 $\pm$ 1.8*
EX + MS	6	27.9 $\pm$ 0.63	245.3 $\pm$ 31.4**	8.72 $\pm$ 1.0**
MS-only	4	24.9 $\pm$ 0.07	144.8 $\pm$ 6.1*	5.90 $\pm$ 0.1*
EX-only	4	30.8 $\pm$ 0.70	258.8 $\pm$ 13.1**	8.65 $\pm$ 1.3**

Values are means $\pm$ SD.

*Significantly lower than normal; **significantly higher than injured control ($p < 0.05$).

Leone, et al., 2002; Mally et al., 2004) has been widely reported. The favorable influence of rTMS stimulation on the nervous tissue may be related to several biochemical changes occurring in exposed nervous tissue. Chronic treatment with rTMS attenuated serotonin uptake, binding to its receptors, and reduced mRNS levels for serotonin transporter (Ikeda et al., 2005). Opposite changes were observed for dopamine and noradrenaline transporters (Ikeda et al., 2005). Interestingly, the changes were similarly expressed in the brain of exposed mice and PC12 cells in vitro. The application of rTMS seems to be especially promising in improving the conditions of Parkinson's patients, attenuating significantly the progress of the disease (Mally et al., 2004). These observations were further supported by research showing that an acute rTMS exposure increased the concentration and release of dopamine in the hippocampus (Ben-Shachar et al., 1997), several frontal structures of the rat brain (Keck et al., 2002; Kanno et al., 2004; Zangen and Hyodo, 2002), and human caudate nucleus (Strafella et al., 2001; 2003). Some of the rTMS-induced effects could be explained by the activation

of specific genes (Schlaepfer and Rupp, 2002), an increase in mRNA expression (Fujiki and Stewart, 1997), and raise in the concentration of cAMP (Hogan and Wieraszko, unpublished). This last observation is especially intriguing considering the pivotal role of the cAMP in neuronal recovery (Pearse et al., 2004). Interestingly, the modifications of the brain functions induced by the exposure to rTMS in vivo are preserved and observed in the tissue in vitro. Long-lasting stimulation of the rat brain with rTMS improved synaptic plasticity in hippocampal slices (Ahmed and Wieraszko, 2006a; Ogiue-Ikeda et al., 2003), made them much more resistant to ischemic damage (Fujiki et al., 2003; Ogiue-Ikeda et al., 2005). The biosynthesis of new molecules induced by rTMS exposure was compellingly demonstrated by Anshel et al. (2003). The cerebrospinal fluid (CSF) obtained from humans exposed to rTMS injected into rats changed their sensitivity to induction of seizures (McNamara et al., 1992). These results support a general notion that exposure to rTMS can induce biosynthesis, and release of new molecules from exposed nervous tissue. These newly synthesized molecules

FIG. 10. Images of the muscle fibers observed in different animal groups. The dark and light colors represent the area with and without muscle fibers, respectively.

may contribute to the better recovery of neuronal functions tested in our behavioral tests. The results show that acrobatic exercises and spinal cord MS complement each other to improve animal scores in BMS and HLT tests. BMS characterizes the gait pattern of the hind limbs as compared to the gait pattern in the normal animals. Therefore, higher BMS scores indicate an improvement in coordination between different muscles, better timing sequencing and scaling of muscle activity, and progress in inter-joints and inter-limbs coordination. The HLT test is even more demanding than BMS, and requires fine sensorimotor balance for the animal to correctly place the hind paw on the grid and maintain it for push off. The ability to maintain the correct strength in the hind limb paw to push off without slipping engages scaling of the muscle activity is a good indicator of enhanced supraspinal connectivity to the lumbar spine. Although the circuitry involved in the locomotion resides locally in the spinal cord, initiation and intensity of activation of these circuitries are supraspinally controlled. While a score of 4 or up in BMS would indicate supraspinally connection to the lumbar spinal cord, HLT score of 15 or less would point out on supraspinal connection especially through corticospinal tracts. The possibility also exists that additional, detour propriospinal pathways would contribute to the observed functional recovery (Courtine, et al 2008).

There was no statistically significant loss in the body weight in any of the groups, although there was a significantly lower, muscle-to-body ration in the MS-only and InC groups. This implies that gastrocnemius muscles atrophied in InC and MS-only groups only. This conclusion supports our previous notion that the functional improvements observed in EX+MS group are the results of the direct stimulation of the spinal cord, and are not due to MS of the muscle. Thus, all these results point towards the exercise as the main factor preventing the atrophy of the muscles. Relatively

FIG. 11. Size of the muscle fibers. (A) Diameter of the muscle fibers calculated from the images shown here was the smallest in injured controls (InC). The diameter of the muscles obtained from exercising animals, exposed to magnetic fields (EX+MS), was approximately 60% of controls, while two remaining groups showed intermediate values. The diameter of the muscles in all groups was significantly lower ($p < 0.05$, one-way analysis of variance [ANOVA] followed by post hoc statistical test) than diameter on the muscles in control group. The diameter of the muscle was significantly larger in EX+MS group than in InC and exercising (EX) ($p < 0.05$, one-way ANOVA followed by post hoc statistical test). X and XX indicate the comparison to normal controls (NC) and InC, respectively. (B) Variability in the diameter of the muscle fibers, expressed as coefficient of variation, was the highest in InC. (C) Volume of the void area, devoid of the muscle tissue, was the largest in InC. Exercising (EX) and exercising exposed animals (EX+MS) demonstrated the void area similar to the control group, while the animals exposed to magnetic stimulation (MS) showed slightly greater void area. These data indicate that our treatment preserved the size as well as the density of muscle fibers. X indicates statistically significant difference, as compared to normal controls (NC; $p < 0.007$) and exercising and exposed (EX+MS) animals ($p < 0.005$), one-way ANOVA ($F = 5.792$, $p = 0.0030$ followed by post hoc Tukey analysis).

diminutive symptoms of recovery observed in Ex animals can be explained by abnormal postural problems (hind-limb crossing, paws fanning and curling) observed in these animals. These postural abnormalities are most likely the result of exercise-induced spasticity, which by itself may prevent muscle atrophy. The spastic muscle characteristics reported in our study agrees with the study by Luke et al. (2006), who reported that the spastic gastrocnemius muscle was found to be atrophied, produce less tetanic force, fatigable, slow and have higher twitch-to-tetanus ratio. We also found that the spastic gastrocnemius muscles were easily fused compared to muscles from normal animals. Remarkably, our treatment strategy (EX+MS) has prevented the development of these spasticity syndrome characteristics. On the other hand, spastic characteristics were noticeably seen in muscles from EX-only animals. Thus, since muscles from EX+MS showed no spastic-like characteristics, MS apparently exerted a protective effect. One of possible factors contributing to this protective effect could be an increase in the concentration of cAMP in the stimulated spinal cord. As previously mentioned rTMS exposure increased the level of cAMP in hippocampal slices (Hogan and Wieraszko, unpublished). Since cAMP facilitates synaptic plasticity (Nguyen, 2003; Balschun, 2003; Kaneko, 2004), and regeneration (Pearse et al., 2004), its elevation could prevent secondary damage to the spinal cord and stimulate recovery of spared, undamaged neurons. But if this would be the case, why did MS-only group not have similar functional improvement as EX+MS? We suggest that the application of the acrobatic exercises is the key to answer that question. The MS, by providing the neuroprotection, would prime the tissue creating the environment which would make neurons more responsive to acrobatic exercise.

A recently published study (Brus-Ramer et al., 2007) showed that electrical stimulation of the pyramidal tract increased axonal sprouting in the spinal cord contralateral to the lesion. Our results are in full agreement with this report. Although we employed magnetic fields to excite the tissue, it was an electrical current which ultimately stimulated axons to generate the action potentials. However, it should be emphasized that while the application of MS used in our study covered the entire spinal cord below and above the lesion, electrical stimulation used by Brus-Ramer and collaborators was very local. It remains to be determined, whether this difference in place of the stimulation has any influence on the functional recovery.

We do not know whether MS caused the spinal cord to be more prone to the beneficial action of the exercise, or if acrobatic exercise created the environment in which MS exerted stronger effects. It is well known that modification in local spinal cord circuitry after SCI is an inevitable process (Rossignol et al., 2008; Vinay and Jean-Xavier, 2008; Harkema 2008). Apparently acrobatic exercise and MS worked synergistically to facilitate the process of modification and recovery and/or slowing down degeneration creating the environment which was more permissive for improvement.

It would be the goal of further research to determine whether our treatment slowed down degenerative processes initiated by SCI, or stimulated regeneration of the spinal cord tissue. In conclusion, our behavioral testing shows significant functional improvement that occurred after combining

the application of spinal MS and acrobatic exercises. These functional improvements are probably due to local as well as distance modification of spinal circuits.

Acknowledgments

This research was supported by PSCCUNY grant #60027-37-38 to Z.A. and PSCCUNY grant # 69255-0038 and NIH 41429 to A.W.

Author Disclosure Statement

No competing financial interests exist.

References

- Ahmed, Z., and Wieraszko, A. (2006a). Modulation of learning and hippocampal, neuronal plasticity by repetitive transcranial magnetic stimulation (rTMS). *Bioelectromagnetics* 27, 288–294.
- Ahmed, Z., and Wieraszko, A. (2006b). The influence of pulsed magnetic fields (PMF) on the synaptic transmission and on the action potential in the mouse nervous system. *Proc. 4th Int. Wkshp. Biol. Effects EMFs* 430–445.
- Anschel, D., Pascual-Leone, A., and Holmes, G.L. (2003). Anti-kindling effect of slow repetitive transcranial magnetic stimulation in rats. *Neurosci. Lett.* 351, 9–12.
- Balschun, D., Wolfer, D.P., Gass, P., Mantamadiotis, T., Welzl, H., Frey, J.U., and Lipp, H.P. (2003). Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory? *J. Neurosci.* 23, 6304–6314.
- Baptiste, D.C., and Fehlings, M.G. (2007). Update on the treatment of spinal cord injury. *Prog. Brain Res.* 161, 217–233.
- Barbeau, H., Basso, M., Behrman, A., and Harkema, S. (2006). Treadmill training after spinal cord injury: good but not better. *Neurology* 67, 1900–1901.
- Barker, A.T. (2002). The history and basic principles of magnetic nerve stimulation, in: *Handbook of Transcranial Magnetic Stimulation*, A. Pascual-Leone, N.J. Davey, J. Rothwell, E.M. Wasserman, and B.K. Puri (eds), A Hodder Arnold Publication: London, pps. 3–17.
- Basso, D.M., Fisher, L.C., Anderson, A.J., Jakeman, L.B., McTigue, D.M., and Popovich, P.G. (2006). Basso Mouse Scale for Locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *J. Neurotrauma* 23, 635–659.
- Belci, M., Catley, M., Husain, M., Frankel, H.L., and Davey, N.J. (2004). Magnetic brain stimulation can improve clinical outcome in incomplete spinal cord injured patients. *Spinal Cord* 42, 417–419.
- Ben-Shachar, D., Belmaker, R.H., Grisau, N., and Klein, E. (1997). Transcranial magnetic stimulation induces alterations in brain monoamines. *J. Neural Transm.* 104, 191–197.
- Bohning, D.E. (2000). Introduction and overview of TMS physics, in: *Transcranial Magnetic Stimulation in Neuropsychiatry*. M.S. George and R.H. Belmaker (eds), American Psychiatric Press: Arlington, VA, pps. 13–44.
- Brus-Ramer, M., Carmel, J.B., Chakrabarty, S., and Martin, J.H. (2007). Electrical stimulation of spared corticospinal axons augments connections with Ips after injury. *J. Neurosci.* 27, 13793–13801.
- Courtine, G., Song, B., Roy, R.R., Zhong, H., Herrmann, J.E., Ao, Y., Qi, J., Edgerton, V.R., and Sofroniew, M.V. (2008). Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nat. Med.* 14, 69–74.

- Crowe, M.J., Sun, Z.P., Battocletti, J.H., Macias, M.Y., Pintar, F.A., and Maiman, D.J. (2003). Exposure to pulsed magnetic fields enhances motor recovery in cats after spinal cord injury. *Spine* 28, 2660–2666.
- Devillard, X., Rimaud, D., Roche, F., and Calmels, P. (2007). Effects of training programs for spinal cord injury. *Ann. Readapt. Med. Phys.* 50, 490–498.
- Dobkin, B., Barbeau, H., Deforge, D., Ditunno, J., Elashoff, R., Apple, D., Basso, M., Behrman, A., Harkema, S., Saulino, M., and Scott, M. (2007). Spinal Cord Injury Locomotor Trial Group. The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: the multicenter randomized Spinal Cord Injury Locomotor Trial. *Neurorehabil. Neural Repair* 21, 25–35.
- George, M.S., and Belmaker, R.H. (2000). *Transcranial Magnetic Stimulation in Neuropsychiatry*. American Psychiatric Press: Arlington, VA.
- Goldshmit, Y., Lythgo, N., Galea, M.P., and Turnley, A.M. (2008). Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery. *J. Neurotrauma* 25, 449–465.
- Fischer, F.R., and Peduzzi, J.D. (2007). Functional recovery in rats with chronic spinal cord injuries after exposure to an enriched environment. *J. Spinal Cord Med.* 30, 147–155.
- Fujiki, M., and Steward, O. (1997). High frequency transcranial magnetic stimulation mimics the effects of ECS in upregulating astroglial gene expression in the murine CNS. *Mol. Brain Res.* 44, 301–308.
- Fujiki, M., Kobayashi, H., Abe, T., and Kamida, T. (2003). Repetitive transcranial magnetic stimulation for protection against delayed neuronal death induced by transient ischemia. *J. Neurosurg.* 99, 1063–1069.
- Harkema, S.J. (2008). Plasticity of interneuronal networks of the functionally isolated human spinal cord. *Brain Res. Rev.* 57, 255–264.
- Hutchinson, K.J., Gómez-Pinilla, F., Crowe, M.J., Ying, Z., and Basso, D.M. (2004). Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. *Brain* 127, 1403–1414.
- Ikeda, T., Kurosawa, M., Uchikawa, C., Kitayama, S., and Nukina, N. (2005). Modulation of monoamine transporter expression and function by repetitive transcranial magnetic stimulation. *Biochem. Biophys. Res. Commun.* 327, 218–224.
- Jones, A.T., Chu, J.C., Grande, A.L., and Gregory D.A. (1999). Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. *J. Neurosci.* 19, 10153–10163.
- Kaneko, M., and Takahashi, T. (2004). Presynaptic mechanism underlying cAMP-dependent synaptic potentiation. *J. Neurosci.* 24, 5202–5208.
- Keck, M.E., Welt, T., Muller, M.B., Erhardt, A., Ohl, F., and Toschi, N. (2002). Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. *Neuropharmacology* 43, 101–109.
- Kim, C.M., Eng, J.J., and Whittaker, M.W. (2004). Level walking and ambulatory capacity in persons with incomplete spinal cord injury relationship with muscle strength. *Spinal Cord* 42, 156–162.
- Krause, P., Edrich, T., and Straube, A. (2004). Lumbar repetitive magnetic stimulation reduces spastic tone increase of the lower limbs. *Spinal Cord* 42, 67–72.
- Luke, R., Harris, W., Bobet, J., Sanelli, L., and Bennett, D.J. (2006). Tail muscles become slow but fatigable in chronic sacral spinal rats with spasticity. *J. Neurophysiol.* 95, 1124–1133.
- Mally, J., Farkas, R., Tothfalusi, L., and Stone, T.W. (2004). Long-term follow-up study with repetitive transcranial magnetic stimulation (rTMS) in Parkinson's disease. *Brain Res. Bull.* 64, 259–263.
- McNamera, J.O., Bonhaus, D.W., and Shin, C. (1992). The kindling model of epilepsy, in: *Epilepsy: Models, Mechanisms and Concepts*. P. Schwartzkroin (ed), Cambridge University Press: New York, pps. 27–47.
- Nahas, Z., Lomarev, M., Roberts, D.R., Shastri, A., Lorberbaum, J.P., and Teneback, C. (2001). Unilateral left prefrontal transcranial magnetic stimulation (TMS) produces intensity-dependent bilateral effects as measured by interleaved BOLD fMRI. *Biol. Psychiatry* 50, 712–720.
- Nguyen, P.V., and Woo, N.H. (2003). Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinase. *Prog. Neurobiol.* 71, 401–437.
- Nichols, T.W., Pearce, L.A., and Bonlie, D.A. (2004). Treatment of peripheral neuropathy with high strength magnetic fields: a preliminary study suggestive of nerve regeneration. *Proc. 3rd Int. Wkshp. Biol. Effects EMFs* 203–208.
- Ogiue-Ikeda, M., Kawato, S., and Uen, S. (2003). The effect of repetitive transcranial magnetic stimulation on long-term potentiation in rat hippocampus depends on stimulus intensity. *Brain Res.* 993, 222–226.
- Ogiue-Ikeda, M., Kawato, S., and Ueno, S. (2005). Acquisition of ischemic tolerance by repetitive transcranial magnetic stimulation in the rat hippocampus. *Brain Res.* 1037, 7–11.
- Orgel, M.G., O'Brien, W.J., and Murray, H.M. (1984). Pulsing electromagnetic field therapy in nerve regeneration: an experimental study in the cat. *Plast. Reconstr. Surg.* 73, 173–183.
- Pascual-Leone, A., Davey, N.J., Rothwell, J., Wasserman, E.M., and Puri, B.K. (eds). (2002). *Handbook of Transcranial Magnetic Stimulation*. Arnold: London.
- Pearse, D.D., Pereira, F.C., Marcillo, A.E., Bates, M.L., Berrocal, Y.A., Filbin, M.T., and Bunge, M.B. (2004). cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. *Nat. Med.* 10, 610–616.
- Poirrier, A.L., Nyssen, Y., Scholtes, F., Multon, S., Rinkin, C., Weber, G., Bouhy, D., Brook, G., Franzen, R., and Schoenen, J. (2004). Repetitive transcranial magnetic stimulation improves open field locomotor recovery after low but not high thoracic spinal cord compression-injury in adult rats. *J. Neurosci. Res.* 75, 253–261.
- Post, A., and Keck, M.E. (2001). Transcranial magnetic stimulation as a therapeutic tool in psychiatry: what do we know about the neurobiological mechanisms? *J. Psychiatr. Res.* 35, 193–215.
- Richardson, P.M., Issa, V.M., and Aguayo, A.J. (1984). Regeneration of long spinal axons in the rat. *J. Neurocytol.* 13, 165–182.
- Rossignol, S., Schwab, M., Schwartz, M., and Fehlings, M.G. (2007). Spinal cord injury: time to move? *J. Neurosci.* 27, 11782–11792.
- Rossignol, S., Barrière, G., Frigon, A., Barthélemy, D., Bouyer, L., Provencher, J., Leblond, A., and Bernard, G. (2008). Plasticity of locomotor sensorimotor interactions after peripheral and/or spinal lesions. *Brain Res. Rev.* 57, 228–240.
- Ruohonen, J., and Ilmoniemi, R.J. (2002). Physical principles for transcranial magnetic stimulation, in: *Handbook of Transcranial Magnetic Stimulation*. A. Pascual-Leone, N.J. Davey, J. Rothwell, E.M. Wasserman, and B.K. Puri (eds), Arnold: London, pps. 18–29.
- Schlaepfer, T., and Rupp, F. (2002). Effects of repetitive transcranial magnetic stimulation on immediate early gene ex-

- pression, in: *Handbook of Transcranial Magnetic Stimulation*. A. Pascual-Leone, N.J. Davey, J. Rothwell, E.M. Wasserman, and B.K. Puri (eds), Arnold: London, pps. 85–89.
- Shah, P.K., Stevens, J.E., Gregory, C.M., Pathare, N.C., Jayaraman, A., Bickel, S.C., Bowden, M., Behrman, A.L., Walter, G.A., Dudley, G.A., and Vandenborne, K. (2006). Lower-extremity muscle cross-sectional area after incomplete spinal cord injury. *Arch. Phys. Med. Rehabil.* 87, 772–778.
- Sisken, B.F., Kanje, M., Lundborg, G., Herbts, E., and Kurtz, W. (1989). Stimulation of the nerve regeneration with pulsed electromagnetic fields. *Brain Res.* 485, 309–316.
- Sisken, B.F., Kanje, M., Lundborg, G., and Kurtz, W. (1990). Pulsed electromagnetic fields stimulate regeneration in vivo and in vitro. *Restor. Neurol. Neurosci.* 1, 303–309.
- Stratella, A., Paus, T., Fraraccio, M., and Dagher, A. (2003). Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. *Brain* 126, 2609–2615.
- Thuret, S., Moon, L.D., and Gage, F.H. (2006). Therapeutic interventions after spinal cord injury. *Nat. Rev. Neurosci.* 7, 628–643.
- Theoret, H., Kobayashi, M., Valero-Cabre, A., and Pascual-Leone, A. (2003). Exploring paradoxical functional facilitation with TMS. *Clin. Neurophys.* 56, 211–219.
- Vinay, L., and Jean-Xavier, C. (2008). Plasticity of spinal cord locomotor networks and contribution of cation-chloride cotransporters. *Brain Res. Rev.* 57, 103–110.
- Walsh, V., and Pascual-Leone, A. (2003). *Transcranial Magnetic Stimulation: A Neurochronometric of Mind*. MIT Press: Cambridge, MA.
- Wassermann, E.M., and Lisanby, S.H. (2001). Therapeutic application of repetitive transcranial magnetic stimulation: a review. *Clin. Neurophysiol.* 112, 1367–1377.
- Wilson, D.H., and Jagadeesh, P. (1976). Experimental regeneration in peripheral nerves and the spinal cord in laboratory animals exposed to a pulsed electromagnetic field. *Paraplegia* 14, 12–20.
- Zangen, A., and Hyodo, K. (2002). Transcranial magnetic stimulation induces increases in extracellular levels of dopamine and glutamate in the nucleus accumbens. *Neuroreport* 13, 2401–2405.

Address reprint requests to:
Zaghloul Ahmed, Ph.D.

CSI/IBR Center for Developmental Neuroscience
Physical Therapy Department
The College of Staten Island/CUNY
2800 Victory Boulevard
Staten Island, NY 10314

E-mail: ahmedz@mail.csi.cuny.edu