1-2006

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Original Article

Restoration of frequency-dependent depression of the H-reflex by passive exercise in spinal rats

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Study design: Hyper-reflexia, measured as a decrease of low frequency-dependent depression of the H-reflex, is known to occur in both humans and animals after spinal cord injury (SCI). Previous studies have shown that passive exercise for 3 months could be used to restore low frequency-dependent depression of the H-reflex after SCI.

Objective: To determine the effects of various periods of time on the ability of passive exercise to restore low frequency-dependent depression of the H-reflex.

Setting: Spinal Cord Injury Mobilization Program of the Center for Translational Neuroscience, the research arm of the Jackson T Stephens Spine & Neuroscience Institute, Little Rock, AR, USA.

Methods: Adult rats underwent complete spinal cord transection at the T10 level. The hindlimbs were passively exercised in different groups of rats for 1 h/day, 5 days/week for 15, 30, 45, 60, or 90 days, and low frequency-dependent depression of the H-reflex was tested.

Results: Statistically significant low frequency-dependent depression of the H-reflex was evident by 30 days of exercise, although numerical reductions were seen even at 15 days. There was a linear decrease in low frequency-dependent depression of the H-reflex with duration of passive exercise.

Conclusions: Passive exercise can restore frequency-dependent depression of spinal reflexes in a time-dependent manner if used following complete spinal transection.

Sponsorship: Supported by USPHS award RR020146 and the Arkansas Biosciences Institute.

Spinal Cord (2006) 44, 28–34. doi:10.1038/sj.sc.3101810; published online 26 July 2005

Keywords: hyper-reflexia; rehabilitation; spasticity; spinal cord injury (SCI)

Introduction

Spinal cord injury (SCI) results in numerous deficits of the motor and sensory systems, including paralysis, anesthesia, and hyper-reflexia below the level of the lesion. Hyper-reflexia is evident in both humans and animals following SCI. The physiological changes that have been postulated to contribute to hyper-reflexia include alpha motoneuron hyperexcitability,¹⁻³ changes in the intrinsic properties of alpha motoneurons,⁴⁻⁷ reduced post-activation depression of transmission from Ia fibers,⁸⁻⁹ synapse growth;¹⁰ alterations in morphology of alpha motoneurons,¹¹ and decreased presynaptic inhibition of Ia terminals.¹²⁻¹⁵ The time course of spinal changes after injury has been proposed to include an early postsynaptic mechanism, possibly involving an increase in excitability and/or receptor upregulation, and a late change involving presynaptic mechanisms possibly involving synaptic growth in spared descending pathways and in reflex pathways.¹⁰ One measure used by numerous investigators to quantify hyper-reflexia is the electrical analogue of the classic tendon jerk reflex, referred to as the Hoffman or H-reflex.²⁻¹⁵ The H-reflex is a compound electromyographic (EMG) response elicited by the synaptic activation of motoneurons by muscle afferents following stimulation of muscle nerves. Thompson et al.¹⁶ investigated four measures of H-reflex excitability in a contusion model of SCI in the rat. Results of their studies led these researchers to conclude that rate-sensitive depression of the H-reflex was of particular importance in the assessment of hyper-reflexia following SCI. Other groups have reached similar conclusions regarding the importance of
changes in H-reflex rate-sensitive depression as a measure of the effects of SCI. Previous studies have demonstrated that exercise can help to prevent or reverse the loss of motor function following spinal cord injury. The effectiveness of exercise therapy has been attributed to its ability to promote neuroplasticity and improve motor function. However, the specific mechanisms underlying these effects are not fully understood.

Methods

Surgery

Adult female Sprague–Dawley rats (Harlan, 200–250 g, n = 40) were used in the study. A total of 80 rats were used, with 20 rats in each of the four treatment groups. The rats were anesthetized with ketamine (60 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) anesthesia. A complete transection (Tx) of the spinal cord was performed by aspiration and the transected ends of the cord were taped together to produce a cavity. Gelfoam was inserted into the cavity to facilitate hemostasis and the dura was closed over the Tx site. Muscle and skin were sutured in separate layers, and animals were maintained in a thermostatically controlled heat lamp. A bipolar cuff electrode was placed on the tibial nerve for stimulation, and a ground electrode was placed on the digits. A ground electrode was attached to the skin of the tail. Recordings were made using amplifier (Grass P511) filter settings of 3 Hz to 3 kHz with the 60 Hz notch filter in use. Responses to the stimulus were digitized and averaged using a GW Instruments digitizer module and SuperScope software.

Exercise

One group of rats (Tx only 90D, n = 4) underwent no further treatment until reflex testing was carried out. Another group of transected animals (Tx only 30D, n = 8) underwent no training but was tested for H-reflex frequency-dependent depression after 30 days, and a group of intact rats served as nontransected controls (CONTROL, n = 5). The remaining rats (n = 23) were divided into five groups that received MBET daily. Exercise was provided for either 15 days (Tx + Ex 15D, n = 5), 30 days (Tx + Ex 30D, n = 4), 45 days (Tx + Ex 45D, n = 5), 60 days (Tx + Ex 60D, n = 5), or 90 days (Tx + Ex 90D, n = 4). The exercise regimen MBET involved suspending the rats on a swing with the hindlimbs hanging down and the hind paws strapped to the pedals of a bicycle-type device, which was driven by a motor. The pedaling motion flexed one hindlimb and simultaneously extended the contralateral one, while avoiding overstressing of either limb. Cycling speed was 30 rpm. Exercise sessions consisted of two 30-min episodes with 10 min of rest in between. After the end of the training period, reflex testing was performed. The two Tx only groups also underwent reflex testing 30 days or 90 days after Tx, along with a group of intact rats (CTL), for comparison with the MBET experimental groups.

Reflex testing

Animals were anesthetized with ketamine (60 mg/kg, i.m.) and maintained with 10% doses as needed such that vibrissal and pinna pinch reflexes were absent. Core body temperature was maintained at 36 ± 1°C using a thermostatically controlled heat lamp. A bipolar cuff electrode was placed on the tibial nerve for stimulation (0.1 ms pulses, cathode proximal on nerve). Exposed tissue was covered with mineral oil to prevent drying. A wire electrode was inserted subcutaneously in the digital interosseous muscles between the fourth and fifth metatarsals for EMG recording as previously demonstrated, and referenced to a clip applied to the skin on the digits. A ground electrode was attached to the skin of the tail. Recordings were made using amplifier (Grass P511) filter settings of 3 Hz to 3 kHz with the 60 Hz notch filter in use. Responses to the stimulus were digitized and averaged using a GW Instruments digitizer module and SuperScope software.

H- and M-wave responses in the rat

Stimulation of the tibial nerve under the calcaneal tendon produced two responses, an early M-wave (~2 ms latency), produced by direct activation of motoneuronal axons in the tibial nerve, and a later H-reflex (~8 ms latency), produced by activation of muscle afferents in the tibial nerve, which synapse monosynaptically on plantar motoneurons. The degree of stimulation that induced frequency-dependent depression of the H-reflex was determined. The reflex was first tested at 0.2 Hz to determine threshold and maximal response levels. After discarding the first five responses in order to obtain an average of the stabilized reflex, averages of 10 responses were obtained. Averages were compiled following stimulation at 0.2, 1, 5, and 10 Hz. The change in the response at various frequencies was calculated as the percent of the response at 0.2 Hz in order to determine depression of the H-reflex as a function of stimulation frequency. Following the frequency series testing, the H-reflex amplitude was confirmed at 0.2 Hz.
for consistency. If the amplitude at recheck was less than 90% of the initial amplitude, the data was discarded.

At the end of the experiment, animals were euthanized with an overdose of barbiturate (Nembutal) and the Tx was confirmed either visually or histologically following transcardial perfusion with paraformaldehyde (4%) and sucrose (20%). Sensory testing or assessment of spasticity was not carried out in this series of animals.

**Measurement and statistics**

The amplitude of the H-wave was measured from the base line before the H-wave to the peak of the first (and largest) of its two components (Figure 1). Measurements from peak to peak of the two components gave similar results so that only the baseline to peak measures are reported here. Results from animal groups were compared statistically using a two-way ANOVA test. Significant differences between groups were tested using the Scheffe test, a conservative post hoc comparison. Statistical significance was considered to be present at $P < 0.05$.

**Results**

Figure 1a shows representative recordings from an intact CONTROL animal stimulated at 0.2 Hz (the 100% response), along with (Figure 1b) the response at 10 Hz (group mean ± SE of the mean, ~11 ± 4% of the response at 0.2 Hz), and the response from a Tx only 60D rat at 0.2 Hz (100%) (Figure 1c), and at 10 Hz for comparison (~68 ± 6%) (Figure 1d). Figure 1e and f show the responses from a Tx + Ex 60D rat at 0.2 Hz (100%) and 10 Hz (~22 ± 7%), respectively. These recordings demonstrate the main effects of higher frequency stimulation leading to marked frequency-dependent depression in intact animals, of Tx leading to decreased (increased percent) frequency-dependent depression, and of MBET leading to a restoration of frequency-dependent depression of the H-reflex.

Figure 2 is a graph of the habituation of the H-reflex following stimulation at 0.2, 1, 5, and 10 Hz in the following groups of animals: Tx only 90D, Tx + Ex 15D, Tx + Ex 30D, Tx + Ex 45D, Tx + Ex 60D, Tx + Ex 90D, Spinal Cord

![Figure 1](image1.png)

**Figure 1** Averages of H-reflex recordings. Evoked muscle potentials included an early M-wave and a later H-reflex following stimulation. (a) Averaged responses to stimulation at 0.2 Hz in a CONTROL rat. (b) Averaged responses to stimulation at 10 Hz in the same CONTROL rat. Note the decrease in amplitude (frequency-dependent depression) of the H-reflex at 10 Hz compared to 0.2 Hz. (c) Averaged responses to stimulation at 0.2 Hz in a Tx only 60D rat. (d) Averaged responses to stimulation at 10 Hz in the same Tx only 60D rat. Note the greater amplitude (decrease in frequency-dependent depression) of the H-reflex at this frequency compared to 10 Hz in the intact animal. (b). (e) Averaged responses to stimulation at 0.2 Hz in a Tx + Ex 60D rat. (f) Averaged responses to stimulation at 10 Hz in the same Tx + Ex 60D rat. Note the return of frequency-dependent depression of the H-reflex after 60 days of MBET. Calibration bars vertical 0.5 mV, horizontal 2 ms.

![Figure 2](image2.png)

**Figure 2** H-reflex amplitude at 0.2, 1, 5, and 10 Hz for intact animals (CONTROL, open circles), and 15 (Tx + Ex 15D, filled diamonds), 30 (Tx + Ex 30D, filled circles), 45 (Tx + Ex 45D, filled triangles), 60 (Tx + Ex 60D, filled plus signs) and 90 (Tx + Ex 90D, filled squares) days of MBET, and 90 days without MBET (Tx ONLY 90D, open squares). Frequency-dependent depression of the H-reflex at 0.2 Hz was designated 100%, and all statistical comparisons in this figure made against the Tx only 90D group. At 1 Hz, the Tx only 90D group (*) differed significantly from the Tx + Ex 45D, Tx + Ex 60D, and CONTROL groups at the $P < 0.05$ level (+); and from the Tx + Ex 30D group at the $P < 0.01$ level (++) at 5 Hz, the Tx only 90D group (*) differed from the Tx + Ex 45D, and Tx + Ex 60D groups at the $P < 0.05$ (+), and was different (**) from the Tx + Ex 30D and CONTROL group at the $P < 0.01$ level (+ +). At 10 Hz, the Tx only 90D group (*) differed from the Tx + Ex 45D group at the $P < 0.05$ level (+), and differed (**) from the Tx + Ex 30D, Tx + Ex 60D, Tx + Ex 90D, and CONTROL groups at the $P < 0.01$ level (+ +).
and CONTROL. The Tx only 30D group was not included because of its similarity with the other longer duration unexercised transected group (Tx only 90D). Statistically significant differences between the Tx only 90D group and the other groups at each frequency are denoted by a single (*$P<0.05$) or a double (**$P<0.01$) asterisk.

ANOVA of these groups showed statistically significant differences across stimulation frequency ($df = 3$, $F = 115.11$, $P<0.001$), across experimental groups ($df = 7$, $F = 6.77$, $P<0.001$), and interaction between frequency and groups ($df = 21$, $F = 2.17$, $P<0.008$). Post hoc comparisons between all groups were undertaken, and those against the Tx only 90D group were considered the most relevant. When comparing this group with the CONTROL animals, there were no significant differences at 1 Hz, but major differences at 5 Hz ($P<0.01$) and 10 Hz ($P<0.01$), indicating decreased frequency-dependent depression (hyper-reflexia) 90 days after Tx. Such decreases in frequency-dependent depression were evident to a similar degree after only 30 days following Tx (Tx only 30D) compared to the CONTROL group ($P<0.01$), suggesting that hyper-reflexia is manifested within 30 days and remains at similar levels in the chronic (90 day) condition.

The effects of MBET for 15 days (Tx + Ex 15D), while producing a numerical reduction of ~15% at each frequency, were not statistically significant. However, after 30 days of MBET (Tx + Ex 30D), there were statistically significant increases in frequency-dependent depression (decreased percent ~30 – %) at all frequencies tested, suggesting that passive exercise led to a restoration of H-reflex habituation. The effects of longer durations of MBET produced decreases in frequency-dependent depression that were variable at the lower frequencies (1 and 5 Hz), but linearly decrementing at 10 Hz. For example, percent frequency-dependent depression decreased from ~29% after 30 days (Tx + Ex 30D), to ~28% after 45 days (Tx + Ex 45D), to ~22% after 60 days (Tx + Ex 60D), to ~7% after 90 days (Tx + Ex 90D) of MBET.

Figure 3 is a comparison of a delay of 30 days following Tx before testing the H-reflex in untrained rats (Tx only 30D) compared to those trained for 30 days after Tx (Tx + Ex 30D) and to intact animals (CONTROL). Basically, ANOVA showed a significant difference across groups ($df = 2$, $F = 12.22$, $P<0.001$) and frequencies ($df = 3$, $F = 50.46$, $P<0.001$), as well as interaction across these two factors ($df = 6$, $F = 2.24$, $P<0.05$). Tx only induced statistically significant decreases in frequency-dependent depression at 5 Hz (**$P<0.01$) and 10 Hz (**$P<0.01$) compared to CONTROL (+ +). MBET for 30 days induced an increase in H-reflex frequency-dependent depression (decreased percent) when comparing the effects of Tx (*$P<0.05$) to Tx + Ex 30D (+ +) at both 5 and 10 Hz. These results show that the decrement in low frequency-dependent depression (1–10 Hz) of the H-reflex induced by Tx can be alleviated by MBET in a period as short as 30 days.

Conclusions

The studies described provide confirmatory evidence that passive exercise in spinally transected animals can be used to restore low frequency-dependent depression of the H-reflex. The decrement in hyper-reflexia was evident in a MBET duration-dependent manner in Tx rats. This methodology has already been shown to induce a similar effect in a spinal cord injured human subject. Before considering these ramifications, a number of issues and potential limitations should be considered.

The H-reflex is a reliable measure of spinal circuitry that is altered after an SCI. This reflex normally undergoes changes with a variety of rhythmic motions such as stepping, walking, running and pedaling. In animals, the H-reflex has been a valuable tool to measure changes in spinal circuitry in both contusion and transection models. The H-reflex has also been used to assess changes in spinal circuitry in the human, a recent effort concluding that (1) chronically paralyzed subjects showed suppression of H-reflexes to...
a lesser extent than able-bodied normals or acutely paralyzed subjects, (2) those with acute paralysis showed similar H-reflex suppression as those within 40 weeks of their SCI, but had decreased frequency-dependent depression after 44 weeks of paralysis (ie there was marked loss of frequency-dependent depression of the H-reflex in the ‘chronic’ condition), and (3) changes in muscle fatigue were associated with a decrease in H-reflex suppression over time.1,13 The results described herein show that frequency-dependent depression of the H-reflex is decreased in the most ‘chronic’ condition tested, 90 days after Tx. Surprisingly, a similar level of hyper-reflexia was evident even at 30 days (Figure 3), suggesting that hyper-reflexia assumes a fairly ‘chronic’ level within 30 days after Tx in the rat. These results are consistent with those of Thompson et al23 who reported significantly less frequency-dependent depression of the monosynaptic reflex at 28 and 60 days postinjury in spinal cord contused rats than in normal animals, but this effect was not evident 6 days after injury. Such results suggest that the onset of low frequency-dependent depression of the H-reflex takes weeks to develop. Further studies are required to determine the time course of the decrement in frequency-dependent depression of the H-reflex in the rat.

Other forms of exercise in animal models also show promise in the ability to effect changes in lower limb function following SCI. For example, adult cats showed recovery of full weight-bearing hindlimb stepping on a treadmill within a few weeks following complete spinal transection if treadmill training was implemented.34,35 Completely spinalized cats that were not treadmill-trained were 3 times less likely to step than those that were trained.36 If spinal cord hemisected rats are immobilized, the expected recovery is delayed and may not be as great (compared to exercised animals) once use is restored.37 We showed that 90 days of MBET minimized loss of muscle mass in transected and exercised, compared to transected only, rats.23,24 Additionally, we showed that MBET restored frequency-dependent depression of the H-reflex to normal levels in exercised, transected rats.24,25 The H-reflex of nonexercised Tx animals failed to show frequency-dependent depression at most stimulation frequencies.

The present studies were designed to determine the time course of recovery induced by MBET on low frequency-dependent depression of the H-reflex. MBET for only 15 days, while not inducing a statistically significant effect on H-reflex habituation, did have a numerical effect, increasing frequency-dependent depression (decreasing percent inhibition) at 10 Hz from 69 to about 42%. MBET for 30, 45, and 60 days induced a similar, significant reduction in percent inhibition from 69 to around 22–29%. Not until 90 days of MBET was there a period that MBET had a fairly rapid ameliorative effect on H-reflex frequency-dependent depression, and could have added effects if continued long term.

Numerous mechanisms have been proposed to account for the hyper-reflexia following SCI, including alpha motoneuron hyperexcitability,1–3 alterations in motoneuronal morphology,11 synapse growth,12 changes in the intrinsic properties of alpha motoneurons,4–7 and decreased presynaptic inhibition of Ia terminals.9,12–15 Decreased presynaptic inhibition is frequently linked with decreased low-frequency depression of the H-reflex.14,21,38 It is thought that the loss of inputs from descending pathways leads to a reorganization of spinal circuitry that promotes hyper-reflexia. MBET induces alternating contractions (via stretch reflexes) of antagonists in the same limb and agonists in different limbs, as previously reported.39 We assume that the cycling motion elicited by MBET provides recurrent signaling that modifies this hyper-reactive circuitry, thereby leading to a restitution of low frequency-dependent depression of the H-reflex.

We have recently found preliminary evidence suggesting an additional potential explanation. Complete Tx in adult rats was found to lead to an increase in neuronal gap junction gene expression.40,41 Gap junctions allow synchronous activation by electrical coupling of spinal motoneurons, but their expression ceases after 7–14 days postnatally.42 Tx thus may lead to an increase in neuronal (specifically Connexin 36) gap junction protein expression. In addition, MBET for 30 days in Tx rats was found to normalize low frequency depression of the H-reflex and to lead to a decrease in Connexin 36 expression.41 We assume that Tx effectively denu- vates motoneurons, which respond by increasing electrical coupling as in the neonatal state (motoneuronal coupling), a condition that fosters paroxysmal co-contraction of agonists, such as is seen in hyper-reflexia and spasticity. MBET may act to decrease electrical coupling by passively forcing alternation leading to motoneuronal uncoupling. Additional studies are required to substantiate and explore this interesting new avenue.

We have also carried out studies on H-reflex frequency-dependent depression in human subjects.28 We tested a total of 15 age- and sex-matched control subjects and six patients with SCI classified as ASIA C (no sensory or motor function below the level of the lesion, 4–8 years postinjury, ie all chronic, > 44 weeks postinjury). Frequency-dependent depression of the H-reflex in control human subjects differed somewhat from that in the intact rat, especially in that frequency-dependent depression at even the highest frequency tested, 20 Hz, was about 10–15% in the human compared to 0% in the rat. The H-reflex in the control subjects decreased to 50–25% of control at frequencies of 1, 3, and 5 Hz, then reached a plateau at this level for higher frequencies. In the SCI patients, frequency-dependent depression of the H-reflex was at 80–65% at 1, 3, 5, and 10 Hz, but decreased closer to normal levels at the higher frequencies tested.28 The use of MBET on one of these SCI subjects led to a normalization of H-reflex frequency-dependent depression within 10 weeks.28 The H-reflex was at 65% at 10 Hz.
in this patient, but was reduced to 15% after 10 weeks of MBET. When MBET was discontinued, there was a gradual return of hyper-reflexia over the next 3 weeks, ultimately returning to previous levels. This suggests that the effects of MBET, at least in the human, (a) require weeks to ensue, and (b) are temporary but last beyond the duration of training, indicating that there is a plastic reorganization in spinal circuitry that can continue to be up or downregulated at the spinal level after SCI. Moreover, there was anecdotal evidence from the subject that spasticity was reduced and bowel and bladder function improved. However, more detailed analyses of these effects are required, along with measures of muscle mass to determine if MBET reduces muscle atrophy in the human as it does in the rat.23

The issue of spasticity requires some discussion. Since spasticity includes both exaggerated reflexes and increased muscle tone, the H-reflex is not considered a selective measure of spasticity, which requires more specific assessment for its quantification.14,43,45 In addition, the rat model is not a good one for spasticity, since rats show variable (to no) levels of spasticity, so that our results cannot address the level of spasticity induced by Tx. Interestingly, the anti-spastic effects of L-dopa were proposed to be mediated by group II, but not group I, afferents,44 suggesting that spasticity involves additional mechanisms to those tested by the H-reflex, which is thought to be mediated mainly by group I afferents. A complementary tool for measuring spastic hypertonia has been proposed for use in humans,44 but such measures in the rat have not been established. Future studies require the quantitative assessment of both flaccid and spastic paralysis to determine if MBET can modulate these sequelae.

Partial weight bearing training (PWBT) has also been utilized in human patients with SCI to effect changes in locomotor function. Improvements in locomotion have been produced in patients with incomplete, but not complete, SCI.56,47 Exercise in the form of PWBT or overground training, combined with functional electrical stimulation (FES),48,49 or pharmacological intervention50 has also yielded promising improvements in locomotor function. However, methods required to assist locomotion as part of these training regimes are labor intensive and physically demanding. More recently developed exercise methods that require fewer personnel to administer, such as the Dietz et al51 are quite expensive and thus fiscally inaccessible for most patients.

The use of MBET for restoring low frequency-dependent depression of reflexes as described here, and the potential for decreasing muscle atrophy after SCI previously described,24,25 suggest that MBET has promise in supplementing or even substituting for some of these techniques. Additionally, MBET can be delivered at low cost to the patient with little physical assistance required. In summary, MBET appears to restore low frequency-dependent depression of the H-reflex in adult Tx rats with at least 30 days of training. A case report of a human subject who underwent MBET supports the contention that such therapy may be beneficial in reducing hyper-reflexia in the human. While a number of parametric studies need to be carried out, MBET appears to be promising for improving the quality of life of SCI victims.

References
Passive exercise in spinal rats

NB Reese et al


