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Enhancement of Functional Recovery Following a Crush Lesion to the Rat Sciatic Nerve by Exposure to Pulsed Electromagnetic Fields

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Previous studies showed that exposure to pulsed electromagnetic fields (PEMF) produced a 22% increase in the axonal regeneration rate during the first 6 days after crush injury in the rat sciatic nerve. We used the same injury model to assess the effect on functional recovery. The animals were treated with whole body exposure to PEMF (0.3 mT, repetition rate 2 Hz) for 4 h/day during Days 1–5 while held in plastic restrainers. Functional recovery was serially assessed up to Postinjury Day 43 using recently described video imaging of the 1–5 toe spread and the gait–stance duration. Footprint analysis was also used with calculation of a sciatic function index. Those animals treated with PEMF had improved functional recovery, as compared to sham controls, using the tests for video 1–5 toe spread and gait–stance duration ($P = 0.001$ and $P = 0.081$, respectively). This effect was found throughout the 43-day recovery period. No effect was found using the sciatic function index. This study confirms that functional recovery after nerve crush lesion is accelerated by PEMF and has broad implications for the clinical use of these fields in the management of nerve injuries. © 1994 Academic Press, Inc.

INTRODUCTION

Regeneration of peripheral nerves can be accelerated by a number of chemical and physical agents including the application of electric (10, 11, 13) and electromagnetic fields (8, 15–17, 19, 21, 22, 26). Clinically, the use of electromagnetic fields applied via Helmholtz coils is particularly attractive since they can be employed noninvasively. Our previous studies using the crushed rat sciatic nerve model with exposure to pulsed electromagnetic fields (PEMF) of 0.3 mT, 2-Hz repetition rate, have shown an increased regeneration rate through the first 6 days (21). Similar increases have been reported using sinusoidal electromagnetic fields (19). When rats were treated with the 2-Hz PEMF signal before crush injury, a pretreatment "conditioning effect" was obtained whereby regeneration was enhanced even when the PEMF exposure was discontinued after injury (9). This phenomenon was not obtained with sinusoidal fields (9) nor has it been reported by investigators working with other low-level electromagnetic signals.

Using a transection model of the sciatic nerve with a 5-day delay in epineural repair, rats exposed to the 2-Hz PEMF signal exhibited significant increases in functional recovery at 165 days as measured by the sciatic function index (26). Earlier studies using this rat model with exposure to Diapulse (a 27-MHz signal, Diapulse Corp., NY) produced an increase in the number and diameter of regenerating axons after 8 weeks and a shorter time for return of the toe spread reflex (17). In addition, exposure of the hind limb of a cat after transection of the common peroneal nerve to a 15-Hz pulse burst signal (currently used by clinicians to enhance bone healing) maintained significantly greater numbers of ventral motor neurons than sham controls (16).

In the present study, we tested longer-term effects of exposure to this 2-Hz PEMF signal after crush injury of the rat sciatic nerve. While our previous studies indicated an enhanced rate of regeneration in the first week after injury with exposure to PEMF, regeneration rate measured at early time periods may not correlate with the desired clinical effect, namely improved long-term function. The purpose of the present study was to determine if this accelerated regeneration resulted in improved functional recovery after crush injury.

METHODS

Animal Model

Adult male Sprague–Dawley rats (200–300 g) were anesthetized using intraperitoneal injection of 65 mg/ml sodium pentobarbital (0.1 ml/200 g body wt). The right sciatic nerve was exposed through the interval between the vastus lateralis and biceps femoris muscles. The sciatic nerve was crushed between the jaws of a 6-in. suture needle holder (Codman 834, Classic Plus 36-3001) at the level of the piriformis tendon just distal to

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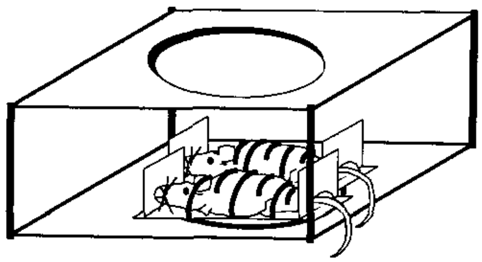

FIG. 1. Schematic drawing of the rats in plastic restrainers between paired Helmholtz coils. The coils were connected to a signal generator (not shown).

the branches to the hamstrings. The needle holder had 1-cm jaws which had been modified by a wire (0.018 in. diameter) fixed longitudinally along the contact area of one of the two jaws to prevent cutting. The crush was applied for 20 s with the jaws closed to the second of three ratchets. This produced a Sunderland Type II injury (axonotmesis). After recovery from anesthesia, the rats were assigned to control or experimental groups. Operations were performed by the same surgeon on four separate dates with four experimental (PEMF) and four sham animals (no PEMF) on each surgical day.

Field Exposure

On Postsurgical Days 1–5, all animals were restrained in plastic restrainers for 4 h/day between paired Helmholtz coils (Fig. 1). The coils were 30 cm in diameter, placed 15 cm apart, and when connected to a signal generator (Bietic Research, Inc., Lyndhurst, NJ) they produced a magnetic field of 0.3 mT with pulse duration of 20 ms and a repetition rate of 2 Hz. Experimental animals were exposed to this field during the restraint period. Sham animals were placed between coils not connected to the generator during the restraint period. While the animals often slept when placed in the restrainers, the restraint period has the potential to induce stress on the animal which could distort physiological effects. Previous experiments with this system have failed to demonstrate any difference in regeneration or recovery between animals which are restrained compared to those allowed cage activity (21, 25). To control for possible effects of restraint in this current study, both sham and experimental animals were restrained in the same way for the same amount of time.

Determination of Functional Recovery

On Postinjury Days 10, 17, 19, 22, 24, 26, 31, and 43 functional recovery was assessed using three different methods: gait–stance duration (25), video 1–5 toe spread (24), and sciatic function index (surgery dates 2, 3, and 4 only) (1). For both the gait–stance duration and video 1–5 toe spread methods, video recording (Panasonic AG160 VHS format) of the plantar aspect of the animal's hind feet was performed during normal walking on a horizontal runway with a frosted Plexiglas surface. The frosting and oblique lighting were used to increase contrast, making the point of foot contact visually more clear. The videotapes were used to find periods of smooth walking defined as at least four consecutive forward motion steps without hesitation. Videotaping occurred at a rate of 60 frames/second. Counting of the frame-by-frame playback was used to determine the duration of floor contact for both the injured and uninjured hind feet. This period of the gait cycle, initial contact to take off, is defined as stance. We defined "gait–stance duration" as the time of this floor contact. The ratio of the injured:uninjured hind feet stance duration was calculated from paired consecutive steps when measured during steady walking. If changes in gait velocity occurred, variability was minimized by pairing the gait–stance duration of each step with the average gait–stance duration from the two steps of the alternate foot immediately preceeding and following it. On each animal, on each assessment day, 4 to 15 such ratios were determined by a single observer blinded to the animal's experimental group assignment.

After acute injury to the sciatic nerve, paralysis of the intrinsic muscles of the foot impairs the rat's ability to spread the toes during walking. To measure this 1–5 toe spread, the videotapes were digitally analyzed using a Kontron Image Processing System IPS X with SEM IPS software. The distance between the first and fifth toes of four injured and four uninjured hindfeet were measured during normal walking. The distances were averaged and a ratio was calculated comparing the injured: uninjured sides. The video 1–5 toe spread measurements were made by a single observer who was blinded to the animal's treatment group assignment.

Walking track analysis has been advocated as a measure of nerve function with combined motor and sensory components used to calculate a sciatic function index (SFI) (1, 5, 7). Tracks were obtained by saturating the plantar aspect of the rat's entire hind feet and all five toes with a fast drying, nontoxic ink and allowing the animal to walk down a runway lined with white paper before the ink dried on the feet. The resultant ink prints were analyzed using the same digital analysis system as above to measure parameters and calculate the SFI as described by Bain *et al.* (1). The prints were measured by a single observer who was blinded to the animal's group assignment and who had previously obtained a normal *Z*-score on the self-evaluation test. (3)

Statistical Analysis

A two-factor analysis of variance (treatment × recovery day) with repeated measures on one factor (recovery day) was run in replicates for each surgical date. Separate analyses were performed for each of the parameters of functional recovery: video 1–5 toe spread, gait–stance

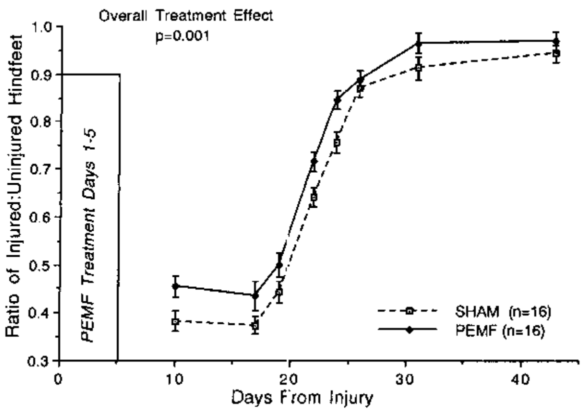

FIG. 2. Effect of PEMF treatment on recovery of the video 1–5 toe spread (mean ± standard error of the mean).

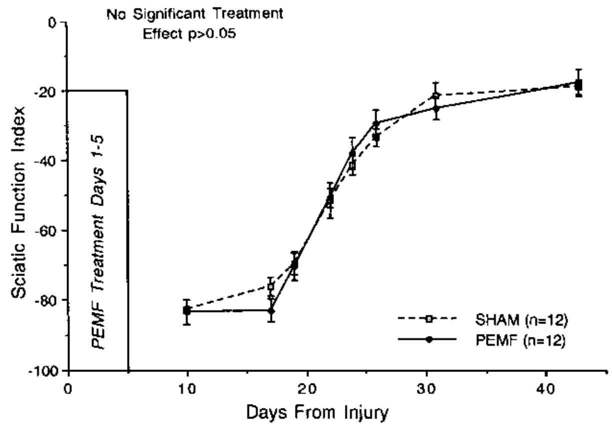

FIG. 4. Effect of PEMF treatment on recovery of sciatic function index (mean ± standard error of the mean).

duration, and SFI. There were four replicates for the video 1–5 toe spread and gait–stance duration tests and three replicates for the SFI method.

RESULTS

The effects of a short (5-day) postinjury treatment with PEMF on each of the parameters of functional recovery following nerve crush in the rat model are shown in Figs. 2–4. Overall ANOVA indicated a PEMF treatment effect as measured by the video 1–5 toe spread ($P = 0.001$; Fig. 2) and of marginal significance by the gait–stance duration ($P = 0.081$; Fig. 3). No effect of PEMF could be detected using the SFI method of functional assessment ($P = 0.76$; Fig. 4). Because there was no interaction found between treatment and recovery day ($P > 0.26$) we conclude that animals treated with PEMF had an enhanced function throughout the 43-day recovery period when compared to sham controls for the video 1–5 toe spread and improved gait–stance duration parameters. Figure 2 indicates that the PEMF enhanced return of the video 1–5 toe spread was greatest in the early stages and during the period of the steepest incline of recovery. Figure 3 indicates that the improved return of the gait–stance duration parameter by PEMF was greatest on Day 43 postinjury compared to shams. As expected, recovery as a function of time varied significantly ($P < 0.0001$) in all parameters of functional recovery.

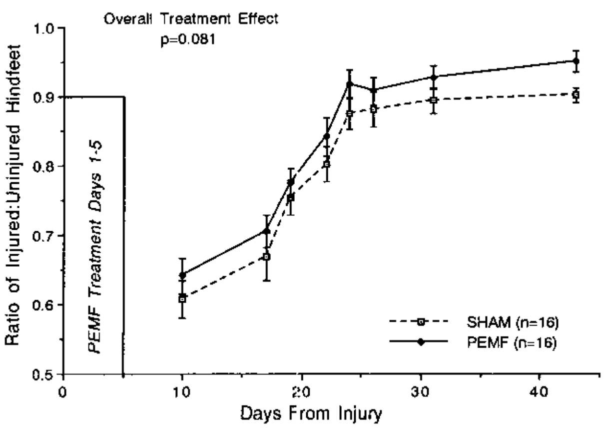

FIG. 3. Effect of PEMF treatment on recovery of gait–stance duration (mean ± standard error of the mean).

DISCUSSION

Short-term exposure of rats, 4 h/day for 5 days while restrained, to PEMF (0.3 mT, 2 Hz) following nerve crush injury enhanced the return of function. The results reported here differ from our preliminary results using only the video 1–5 toe spread which indicated no PEMF effects (23). This current report contains the data of further testing of more animals/group and demonstrates a more rapid return of function by exposure to PEMF. Significant enhancement in the rate of early functional recovery was found using the video 1–5 toe spread measurement and an improvement in recovery of the gait–stance duration measurement. No effect of PEMF was detected using the SFI method. The SFI method, despite having a high correlation with the recovery of video 1–5 toe spread and gait–stance duration parameters, has greater interanimal variability than these other methods (24, 25). Other authors have found that the SFI is a fairly insensitive method for detecting changes in functional recovery (6, 12, 20). This demonstrates the need for developing other more sensitive, standardized tests for measuring return of function in this animal model.

The two methods developed in our laboratory, video 1–5 toe spread and gait–stance duration, using fairly sophisticated recording and imaging techniques are capable of detecting small changes in recovery. These two methods indicate positive effects (up to 43 days postinjury) from noninvasive PEMF applied immediately after injury for only 5 days. The 22% increase in regeneration rate found by Sisken *et al.* (21) at 3, 4, and 6 days after crush lesion may translate to the higher level of functional recovery seen in this present study throughout the recovery period using both the video 1–5 toe spread and the gait–stance duration methods. Increased long-term healing rates have also been reported for bone (2, 18) and skin (4) with PEMF. These higher nerve regeneration rates and acceleration of functional recovery have broad implications for clinical use in the management of nerve injuries. The mechanisms by which pulsed electromagnetic fields exert their enhancing effects on nerve regeneration are unknown and are currently under investigation in a separate series of experiments.

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