Effect of Pulsed Electromagnetic Stimulation on Facial Nerve Regeneration

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**Objective:** To determine if exposure to electromagnetic fields influences regeneration of the transected facial nerve in the rat.

**Design and Methods:** The left facial nerve was transected in the tympanic section of the fallopian canal in 24 rats randomly assigned to 2 groups. The cut ends of the facial nerve were reapproximated without sutures within the fallopian canal to maximize the potential for regeneration. Rats in the experimental group (n=12) were then exposed to pulsed electromagnetic stimulation (0.4 millitesla at 120 Hz) for 4 hours per day, 5 days per week, for 8 weeks. Rats in the control group (n=12) were handled in an identical manner without pulsed electromagnetic stimulation. Four other rats were given sham operations in which all surgical procedures were carried out except for the actual nerve transection. Two of these rats were placed in each group. Nerve regeneration was evaluated using electroneurography (compound action potentials), force of whisker and eyelid movements, and voluntary facial movements before and at 2-week intervals after transection. Histological evaluation was performed at 10 weeks after transection. Each dependent variable was analyzed using a 2-way analysis of variance with 1 between variable (groups) and 1 within repeated measures variable (days after transection).

**Results:** Statistical analysis indicated that N1 (the negative deflection of depolarization phase of the muscle and/or nerve fibers) area, N1 amplitude, and N1 duration, as well as absolute amplitude of the compound action potentials, were all significantly greater 2 weeks after transection in the experimental than in the control group of rats. The force of eye and whisker movements after electrical stimulation was statistically greater in the experimental group of rats 4 weeks after transection. Voluntary eye movements in the experimental group were significantly better at 5 and 10 weeks, while whisker movements were better at 3 and 10 weeks. There was no statistical difference between the 2 groups for any histological variable.

**Conclusion:** Results of this study indicate that pulsed electromagnetic stimulation enhances early regeneration of the transected facial nerve in rats.


Biological stimulation by electromagnetic field exposures can modify cellular functions in bone and nervous tissue, and evidence is accumulating that the regeneration capacity of the tissue may be affected. For example, selective changes in levels of calcium, cyclic adenosine monophosphate, the synthesis of collagen and proteoglycans, DNA, and RNA have been demonstrated in osseous, nervous, and mesenchymal tissue. Pulsed electromagnetic fields have encouraged healing of fractured bones and benefited reanastomosis of peripheral nerves after transection. Wilson and Jagadeesh transected the ulnar nerve in rats and then applied pulsed electromagnetic stimulation for 15 minutes per day for 30 days while rats receiving the lowest stimulation showed return of conduction only after 60 days. Ito and Bassett transected the sciatic nerve in rats and found a significant difference histologically and functionally at 4 weeks, with no difference in the 12- vs 24-hours per day stimulation time. Raji and Bowden also performed axotomy and immediate repair of the common peroneal nerve in rats. High-peak pulsed electromagnetic stimulation was delivered for 15 minutes per day at 3-day and 1-, 2-, 3-, 4-, and 8-week intervals. In the treated group, the time to functional recovery, as determined by leg use and the toe-spread reflex, was significantly quicker than in the control group at 12 and 21 days. Additionally, the pulsed electromagnetic stimulation group had less epineural thickening, less intraneural edema, increased size of intraneural blood vessels, and enhanced axonal regeneration. The use of pulsed electromagnetic stimulation in the management of peripheral nerve injuries warrants additional research.
MATERIALS AND METHODS

ENOG STANDARDIZATION

The technique of surface-recorded electroneurography (ENOG) was first standardized using 10 male Sprague-Dawley rats weighing about 300 g. Institutional guidelines regarding animal experimentation were followed and all protocols were approved by the University of Oklahoma Institutional Animal Care and Use Committee. All animals were anesthetized with ketamine hydrochloride, 100 mg/kg, and xylazine hydrochloride, 3 mg/kg, intramuscularly. The preauricular and postauricular hair and the lower facial region just posterior to the whiskers were clipped and then chemically depilated. Surface stimulation of the facial nerve lying deep within the tissue was performed at the lower attachment of the auricle using a ball-tipped bipolar stimulating electrode with an interelectrode distance of 5 mm. Skin resistance at both the stimulating and recording site was reduced to less than 5000 kohms using electroconductive gel. The stimulation electrode was held and adjusted with a micromanipulator until slight facial movement was detected. The stimulation was trained at a rate of 1 Hz for 10 seconds (10 CAPs) with an intensity range of 2 to 5 mA, depending on the threshold of facial movement. A similar bipolar electrode was used to record the evoked activity; it was placed along the posterior edge of the whiskers and adjusted until the maximal CAP was obtained (Figure 1). The CAP was amplified (Grass model 7BA, Grass Instruments, West Warwick, RI) and recorded using a videocassette recorder-digitizing device (model DR 886, NeuroData Instruments Corp, NeuroData Inc, New York, NY). Before removing, the positions of the stimulating and recording electrodes were marked, subdermally, with a 26-gauge needle filled with india ink. One additional 10-second recording (10 CAPs) was performed after replacing the electrodes onto the marked sites. The morphological characteristics of the CAP, including the area under the curve of N1 (the negative deflection of depolarization phase of the muscle and/or nerve fibers), N1 amplitude, N1 duration, N1 latency, onset latency, and the absolute amplitude, were evaluated as the mean of 10 CAPs (Figure 1). Calculations were performed using waveform analysis software (SPIKE H, Cambridge Electronic Design, Cambridge, England). The repeatability of the technique was then determined by comparing the mean values of the 2 test sessions with a dependent t test (P < .05).

In 3 rats, facial nerve and ENOG recordings were made every 6 hours for 72 hours after transection to determine whether the facial nerve surgery created a complete transection.

FORCE OF WHISKER MOVEMENT

AND EYE CLOSURE TECHNIQUE

AND STANDARDIZATION

A new technique was designed to measure the function and strength of facial movement. Ten male Sprague-Dawley rats weighing about 300 g were used to determine the stimulation parameters necessary to obtain tetany (maximum contraction) in the rat facial muscles and the resulting force generated by the whiskers and eye closure at the threshold of tetany. The rat’s head was stabilized in a position to place the whiskers in the horizontal plane. The bipolar stimulating electrode used for the ENOG experiment stimulated the facial nerve at the same ink-marked site. An L-shaped metal probe was used to interface the whiskers to the transducer. Two adjacent whiskers were attached to the short portion of the probe with collodium and the long portion of the probe was connected to the transducer. This allowed the orientation of the whisker movement to be perpendicular to the transducer, resulting in the most sensitive configuration for the whiskers to exert force onto the transducer. An additional L-shaped metal probe was attached to the upper eyelashes with collodium and oriented perpendicular to the closure of the eyelid. Both probes were connected to force displacement transducers (Grass model FT03, Grass Instruments), which gave a force rate of 0.05 kg/min. The transducers were connected to micromanipulators to orient the probes precisely onto the eyelashes and whiskers. The 2 transducer wires were then connected to a whetstone bridge (Grass model 7P122) and the force displacement was measured in volts using an oscilloscope. The sensitivity of the 2 bridges was adjusted so the force of displacement was less than 5 V. The rats were then stimulated for 5 seconds (1-microsecond square wave, 4-6 mA, at 60-90 Hz). Tetany was determined both by subjective observation of complete facial contraction and by maximum force transducer amplitude. These stimulation parameters were determined by trial and error with several rats to find the general range needed to produce tetany. Identical stimulation parameters were repeated after the probes had been removed and reattached in the same positions. The maximal amplitude of force and area under the curve were recorded and calculated as before (Figure 2).

FACIAL NERVE REGENERATION EXPERIMENT

Twenty-eight male Sprague-Dawley rats were randomly assigned to 2 groups: an experimental group that underwent facial nerve transection and received pulsed electromagnetic stimulation (12 rats) and a control group that also underwent facial nerve transection but received no electromagnetic stimulation (12 rats). The 4 remaining rats received sham operations including all procedures except for the actual nerve transection. Two of these rats were placed into each group. The pulsed electromagnetic stimulation was delivered via 4 custom-made cages equipped with Helmholtz coils. The cages were constructed from polyvinyl chloride (PVC) pipe (30.48-cm diameter) cut to 61-cm lengths that were wrapped on the outer circumference with 75 turns of 24-gauge copper coated wire in 2 tightly bound bundles. The 2 bundles were placed 15.24 cm apart (half the diameter of the cylinder) and connected to each other by a single wire that was continuous from the upper bundle to the lower vessels, and more mature myelination. Cordeiro et al10 transected the sciatic nerve in rats and used a nerve guide to bridge a 5-mm separation in the anastomosis. The rats were placed in high-energy static electromagnetic fields for 12 hours per day for 4 weeks. No difference was found in myelinated axon counts or in the latency of amplitude of the compound action potentials (CAPs) between treated and untreated rats. Orgel et al11 studied regeneration of the common peroneal nerve in cats during exposure to pulse-burst electromagnetic fields for
10 hours per day for 12 weeks. Although no nerve CAPs reached preoperative values, treated muscle CAPs did approach preoperative values. There was statistically significant improvement in labeling and localization of anterior horn cells in the central nervous system with the pulse-burst electromagnetic stimulation, but functional return was not examined.

Taken together, the results of these studies suggest that pulsed electromagnetic stimulation can enhance the reparative process of injured nerves. However, there is
no direct evidence of this process for the transected facial nerve. Therefore, our study was designed to directly measure the effect of pulsed electromagnetic stimulation on regeneration in rats with surgically transected facial nerves.

**RESULTS**

**ENOG STANDARDIZATION**

Using a dependent t test, the standardization of the ENOG data using absolute amplitude ($t=1.72; P>0.05; n=11$) and N1 amplitude ($t=0.95; P>0.05; n=11$) showed no significant difference between repeated recordings. After facial nerve transection, the ENOG waveform was not recordable at 38 to 48 hours and no masseter muscle artifact could be recorded at the threshold level using the preoperative stimulation levels; no facial movement could be observed.

**FORCE STANDARDIZATION**

Using a dependent t test, the standardization of the force resulted in no significant difference between recordings when the force transducers were removed and then replaced on the same whiskers and upper eyelashes, measuring both the maximum amplitude ($t=0.54; P>0.05; n=6$) and the area under the curve ($t=0.12; P>0.05; n=6$).

**NERVE TRANSECTION EXPERIMENT**

The electromagnetic fields were relatively uniform within each cage and between the 4 cages as measured with the gaussometer. Due to amplifier failure, there was a 3-day period during the second week in which the rats were not placed into the cages to allow time to obtain and change power amplifiers. There were no wound infections in any of the rats, but there was 1 death of unknown cause in the experimental group during the sixth week. All rats gained weight throughout the experiment, with no statistical difference between the groups ($P>0.05$).

There was no significant difference at time 0 (preoperatively) between the experimental and control groups for any of the ENOG variables. Only at the 2-week postoperative interval were there statistically significant differences between the 2 groups (Figure 4). The experimental group showed an increase in the N1 area, amplitude, duration, and absolute amplitude ($P<0.05$). There was no difference in N1 latency or onset latency. The rats that underwent sham operations showed no difference in the ENOG recordings between the normal side and transected side, or between the experimental and control groups either preoperatively or 2 and 8 weeks postoperatively.

Return of electrical activity was calculated as a percentage of the normal uncut nerve. The experimental to control group returns were 47% to 12% at 2 weeks; 51% to 39% at 4 weeks; 82% to 49% at 6 weeks; and 91% to 66% at 8 weeks. There was no significant difference between the groups in the rats that underwent sham operations ($P>0.05$).

Mean ($\pm$SD) force of eye closure for the experimental and control groups was statistically significant ($P<0.05$) at the 4-week interval only: $1.38\pm0.30$ V and $0.47\pm0.19$ V, respectively (Figure 5). The mean ($\pm$SD) integrated

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**Figure 1.** The myogenic compound action potential showing the parameters measured after electrical stimulation of the skin surface. N1 indicates the negative deflection of depolarization phase of the muscle and nerve fibers.

**Figure 2.** The output curve generated by a force transducer attached either to the rat’s whiskers or eyelashes to measure facial movement of the nose or eye regions, respectively.

**Figure 3.** Rats were placed into cages that produced electromagnetic field stimulation through 2 Helmholtz coils. One coil was wrapped around the circumference of the cage above the animal and one below. The 2 coils were located half the diameter of each coil apart.
area under the curve (force × time) for the experimental group was 579 ± 184 mm²; for the control group, 96 ± 56 mm² (P < .05) (Figure 4).

Mean (±SD) force of whisker movement for the experimental and control groups was statistically significant (P < .05) only at the 4-week interval as well: 1.26 ± 0.24 V and 0.41 ± 0.13 V, respectively (Figure 6). However, there was no difference in the integrated area under the curve.

As for the clinical assessment of facial movements using the subjective scale, whisker movement in the experimental group was significantly better at 3 and 10 weeks and eye closure was better at 5 and 10 weeks (Figure 7). There was no difference in the intraobserver ratings.

The histological examinations showed no difference in the size of axons or the nerve, number of axons, or myelin count between the experimental and control groups. Comparing the normal uncut nerve with the regenerated cut side, the normal nerve had larger but fewer axons.

**COMMENT**

Although there has been little conclusive proof that pulsed electromagnetic fields enhance or alter nerve regeneration, there is growing evidence to support this conclusion. We believe the results of this study provide experimental support for the beneficial effects of pulsed electromagnetic stimulation in the early regeneration of the facial nerve in rats.

In our study, improvement in facial nerve function after complete transection was facilitated under the influence of pulsed electromagnetic fields compared with controls. This facilitation was objectively measured electrophysiologically and behaviorally. Nerve conduction improved during the first 2 to 4 weeks, followed by physiologic improvements in eye and whisker movements at...
The length of the experiment (8 weeks) was thought to be adequate time for the rat facial nerve to regenerate, even with no enhancing effects. The rat generally re-
ceived no pulsed electromagnetic stimulation. It was, however, not stimulated at the threshold of facial movement at which the ENOG recordings were made. Evidence that does not support a theory of hyperexcitability comes from the recordings of the uninjured facial nerves and muscles. The normal right sides and the rats undergoing sham operations actually showed a trend toward decreased CAPs throughout the experiment. Even though the differences were not significant, the pulsed electromagnetic stimulation certainly was not causing a trend of any type toward increasing the CAP in the uninjured nerve. Additionally, results in the rats that received sham operations showed that making an incision was also not a factor in causing hyperexcitability in the presence of pulsed electromagnetic stimulation.

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