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.

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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 12, 30, 45, or 60 days. Rats receiving the highest stimulation had some return of nerve conduction at 30 days while rats receiving the lowest stimulation showed return of conduction only after 60 days. Ito and Basset 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
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 kilohms 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 Info, 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 with the outer circumference with 73 turns of 24-gauge cooper 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
A platform was fashioned inside the cage at the level of the lower bundle. A smaller diameter (25-cm) PVC pipe was placed on the platform inside the cage to confine the rats away from the walls of the cage and thus the higher intensity magnetic field. A clear plastic plate with multiple large air holes was placed on top of the inside PVC pipe so the rats were completely confined between both the coils. The electromagnetic field within this space was measured with a gaussmeter. The electromagnetic field was produced by a 120-Hz sine wave (Wavetek function generator model 182, Wavetek, San Diego, Calif) and led to a stereo amplifier (Sony model STR-V25, Sony, Park Ridge, NJ, or Adcom model GFA-545, Adcom, East Brunswick, NJ). The output of the amplifier was adjusted to create a 0.4-millitesla magnetic field as measured with the gaussmeter in parallel to each of the 4 cages. The 0.4-millitesla field was calibrated with a search coil and oscilloscope so that it could easily be checked at the beginning and end of each day’s experiment. The cages were placed in a low-light sound booth with ventilation.

All rats were anesthetized and a postauricular incision was made to avoid the skin surface stimulation sites at the base of the attachment of the auricle. After cutting through the cranial portion of the platysma and the levator auris longus muscles, an easily identifiable place along the anterior border of the clavotrapezius muscle was established. With the clavotrapezius muscle retracted posteriorly and the auricle retracted anteriorly, the facial nerve was identified exiting the stylomastoid foramen. The external auditory canal, located immediately anteriorly and superiorly to the nerve, was entered, thus exposing the tympanic membrane. The tympanic membrane was opened and the malleus and incus removed. The superior tympanic bone was gently curetted to allow better exposure of the tympanic portion of the fallopian canal. Using a gently curved pick, the fallopian canal was opened directly superior to the stapes where the stapedial artery had completely diverged from the canal. A 1-mm right-angle pick was used to remove approximately 2 to 3 mm of bone off the lateral face of the canal, thus exposing the entire lateral aspect of the facial nerve. The facial nerve was transected in a single cut with a curved scalp knife and the nerve ends were exposed to visually confirm a complete transection. The nerve was laid back into the fallopian canal, taking care that the 2 ends with the epineurium were well approximated. The postauricular muscles and skin were then closed with interrupted 4-0 polyglactin 910 sutures.

The rats were allowed to recover overnight, and the following day each was randomly assigned to the experimental or control group. On each subsequent day, each rat was randomly placed into 1 of the 4 cages, with 3 rats to a cage for 4 hours per day and 5 days per week. The magnetic field was present for the experimental but not for the control group. Each week, the time of day that each group was in the cage was reversed; therefore, the pulsed electromagnetic stimulation group would spend 4 hours in the morning one week and 4 hours in the afternoon the following week in the cage. The experiment was carried out for 8 weeks. Food and water ad libitum were provided throughout. The weight of each rat was recorded every 2 weeks.

Electroneurography was performed on both the operative (left) and the normal (right) sides preoperatively and at 2-, 4-, 6-, and 8-week intervals following transection. The force of whisker movement and eye closure was measured at the same time and at the same intervals on the operative side only. The percentage of reduction in the CAPs comparing the operative and the normal sides was calculated with the following formula: 100−(amplitude of operative side/amplitude of normal side) × 100.

A subjective scale of facial movement was designed to assess return of behavioral motion from no movement (0%) through partial movement and substantial movement (25%, 50%, and 75%) to complete movement (100%). Eye closure (blink) was assessed, similarly, by the corneal reflex from no closure (0%) through partial and substantial (25%, 50%, and 75%) to complete closure (100%). These scales were used by 3 independent and blinded observers at 1-, 2-, 3-, 5-, 8-, and 10-week periods during the experiment.

A histological examination was performed at 10 weeks on both the normal and operative sides from 2 of the best functioning rats and 2 of the worst functioning rats with regard to facial movement from both the experimental and control groups. A segment of the facial nerve was removed from the temporal bone by transecting the nerve proximal to the original lesion and removing the nerve segment to the stylomastoid foramen. These segments were pinned to a wax sheet with the proximal end superior and fixed in 10% buffered formalin. They were dehydrated and embedded in paraffin. Serial sections were cut in a plane perpendicular to the long axis of the nerve at 10-µm thickness. Every 50th section was mounted and stained with a modified Bodian stain (dark brown axons) and counterstained with Luxol fast blue (blue myelin sheaths). With high-power light microscopy (×1200) (Olympus Vanox-S, Olympus America Inc, Melville, NY) and computerized image analysis (Microcomp, Southern Micro Instruments Inc, Atlanta, Ga), the axons were counted for 3 sections of the nerve and averaged. The amount of myelin was determined based on a subtraction percentage of the background and axon staining. Empty endoneurial tubes were also counted. Data were compared with the normal side and between rats in the experimental and control groups.

**STATISTICAL ANALYSIS**

Statistical analysis was performed on all collected data. The standardization tests were evaluated using t tests and basic descriptive statistics. The ENOG and force data were analyzed using 2-way analysis of variance with 1 between variable (groups) and 1 within repeated measures variable (time after transection). Post hoc analysis after significant main and interaction effects was performed with the Newman-Keuls test. The behavioral functional return was evaluated by the nonparametric Kruskal-Wallis analysis of variance.
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 (±SD) force of eye closure for the experimental group was statistically significant ($P<0.05$) at the 4-week interval only: 1.38±0.30 V and 0.47±0.19 V, respectively (Figure 5). The mean (±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.

**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.

**Figure 5.** Mean (±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±0.30 V and 0.47±0.19 V, respectively. The mean (±SD) integrated
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
4 weeks, and finally by behavioral observations of eye and whisker movements at 3 to 10 weeks. It must be mentioned that although use of pulsed electromagnetic fields improved the electrophysiological measurements during the early course of facial improvement, the control group had improved as much by the end of the experiment (8 weeks). Thus, although it is clear that there is a positive effect with the early use of pulsed electromagnetic fields, differences beyond 8 weeks may not have been recognized because of lack of sensitivity in the measurement. Additionally, the lack of detailed facial movement of the rat may have masked visual movement differences even after the electrical depolarization and repolarization and strength of movement had equilibrated in the 2 groups. Despite this potential problem, the trained observers in this study could still detect a difference in facial movement at 10 weeks in the rats receiving pulsed electromagnetic stimulation.

During the design and execution of this experiment, the utmost care was taken to ensure that the experimental and control groups were treated exactly the same except for the delivery of pulsed electromagnetic stimulation. A balanced design was used so that all rats spent equal time in all the cages and equal time in the morning and afternoon sessions. The time of day each group was entered into the experiment was alternated so daily circadian rhythms with their corresponding physiological changes in hormones would not be a confounding factor in nerve regeneration. No difference was observed in the behavior or activity after each day of the experiment between the 2 groups.

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 regenerates nerves at a rate of approximately 3 to 4 mm/d. The facial nerve in a 300- to 400-g rat is about 3 to 4 cm from the region of the transection to the facial musculature. Thus, after wallerian degeneration has occurred, nerve regeneration should take place in approximately 2 weeks. This seemed to be the case from an electrical standpoint, with better regeneration in the pulsed electromagnetic stimulation group. Functionally, the nerve was not able to be stimulated sufficiently to contract the facial muscles until 4 weeks. The behavioral return of function was not completed in most rats by 10 weeks, but may have been the maximum amount of return that would occur.

The transection in the middle ear at the tympanic segment provided the advantage of a complete nerve transection proximal to all the branches and for the best possible realignment of the nerve with the least variability. The nerve was completely cut through in one move and placed back into correct anatomical alignment in the fallopian canal with little manipulation trauma. This technique also preserved the skin area inferior to the auricle for the ENOG and force measurement stimulations.

The proper and minimum intensity of the electromagnetic field to produce nerve regeneration has yet to be determined. The most important factor seems to be not the intensity, but rather the fact that it is pulsed. The experiments using static electrical or electromagnetic stimulation showed no difference in nerve regeneration. It is theorized that the changing polarity of the current alters the nerve electrochemical environment, favoring better axoplasmic flow, more effective enzymatic activity for protein synthesis, and/or inhibition of fibrosis at the injury site. The phagocytic activity of the Schwann cells may also be enhanced, and these cells also have some control over nerve growth factor release. The pulsed electromagnetic stimulation may also lessen the degree of nerve cell body injury during the degeneration phase of injury, along with increasing the protein synthesis during regeneration.12

It is unclear if the pulse frequency or the type of wave makes a difference. This experiment used a sinusoidal wave at 120 Hz. Other experiments have used both a sinusoidal wave or a narrow (short burst) square wave at various frequencies from 2 to 72 Hz with positive nerve regeneration effects.

Positive effects were demonstrated in the myogenic CAPs at 2 weeks. The CAP is the recording of muscle membrane depolarization and depends on the product of the number and diameter of the motor fibers generating action potentials. In this study, we interpreted the experimental group as having more motor fibers and/or larger diameter fibers generating action potentials, thus creating a larger recorded voltage of depolarization and repolarization compared with that in the control group. This, in turn, indicates better neural integrity of the facial nerve. Perhaps the pulsed electromagnetic stimulation in the experimental group caused the facial muscle fibers to be more sensitive or hyperactive, thus causing a direct muscle excitation from the stimulation site. Another possibility is that the masseter muscle became more hyperactive in the experimental group, causing an artifactual recording with stimulation of the facial nerve that was not present in the preoperative recordings or in the control group that 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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