Effect of Pulsed Electromagnetic Stimulation on Facial Nerve Regeneration

John M. Byers, MD; Keith F. Clark, MD; Glenn C. Thompson, PhD

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.
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 ohms using conductive 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 collodion 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 collodion 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 73 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
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

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.

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

ENOGR STANDARDIZATION

Using a dependent t test, the standardization of the ENOG data using absolute amplitude (t=1.72; P>.05; n=11) and N1 amplitude (t=0.95; P>.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>.05; n=6) and the area under the curve (t=0.12; P>.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>.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<.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>.05).

Mean (±SD) force of eye closure for the experimental and control groups was statistically significant (P<.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

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<.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<.05) at the 4-week interval only: 1.38±0.30 V and 0.47±0.19 V, respectively (Figure 5).
The area under the curve (force × time) for the experimental group was 579±184 mm²; for the control group, 96±56 mm² (P < 0.05) (Figure 4).

Mean (±SD) force of whisker movement for the experimental and control groups was statistically significant (P < 0.05) 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 rat generally reserved in the behavior or activity after each day of the stimulating factor in nerve regeneration. No difference was observed in hormones would not be a confounding factor in nerve regeneration. 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 received no 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 field (pemf) stimulation at 4 and 10 weeks. Whisker movements were judged to be better at 3 and 10 weeks. Asterisk indicates P<.05.

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 received no 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.
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.

Accepted for publication November 5, 1997.
Presented at the Society of Head and Neck Surgery combined spring meetings, Palm Beach, Fla, May 10, 1994.
Reprints: John M. Byers, MD, 321 W Wendover Ave, Greensboro, NC 27408.

REFERENCES

7. Wilson DH, Jagadeesh P. Experimental regeneration in peripheral nerves and the spinal cord in laboratory animals exposed to a pulsed electromagnetic field. Paraplegia. 1976;14:12-20.