Daily Facial Stimulation to Improve Recovery After Facial Nerve Repair in Rats

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Objective: To establish whether daily mechanical stimulation improves functional recovery of whisking after facial nerve transection injury and repair in rats.

Methods: Forty rats underwent facial nerve transection injury and repair and subsequent quantitative facial movement testing. Animals were randomized into 2 experimental groups (n=20 each). Both groups received daily 5-minute manual stimulation of their whiskers, with one group undergoing whisker protraction and the other, whisker retraction. Rats were tested on postoperative weeks 1, 4 through 8, and 15 via a validated, quantitative whisking kinematics apparatus. Whisks were counted and analyzed for whisking amplitude, velocity, and acceleration.

Results: Animals receiving manual stimulation by passive protraction of their whiskers demonstrated significantly improved functional recovery at multiple time points during the 15 weeks compared with historical controls (P<.005; 1-tailed t test). Recovery was similar in the protraction and retraction groups, trending toward better whisking recovery in the protraction group.

Conclusions: Daily mechanical whisker stimulation via either protraction or retraction significantly improves recovery of whisking after facial nerve transection and repair. This finding supports the role of early soft-tissue manipulation after facial nerve repair and may have clinical implications for the postoperative management of patients after facial nerve manipulations.

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Facial paralysis is a clinical disorder that carries significant adverse social and functional consequences, including decreased ability to communicate using facial expression, incomplete eye closure, external nasal valve collapse, and oral incompetence. Clinically, many studies have demonstrated poor functional recovery after facial nerve transection injury and microsurgical repair.1-5 The slow rate of facial nerve regeneration following certain injury scenarios can lead to degeneration of the motor end organ and permanent loss of function. Recognizing this, many researchers have used animal models to study manipulations that might accelerate recovery, using qualitative and semiquantitative methods to measure facial recovery after injury.6-8 A variety of pharmacologic agents have been shown to improve motor nerve regeneration in animals, including FK-506,8 TJ-23,9 angiotensin II,10 nitric oxide,11,12 and brain-derived neurotropic factor13; however, owing to adverse effects and the difficulties with drug delivery and bioavailability, none of these drugs is in clinical use. Likewise, some nonpharmacologic treatments have shown benefit. Recently, manual mechanical stimulation of paralyzed facial14 and tongue15 musculature has demonstrated promise as a possible treatment option following cranial nerve transection. Despite a wealth of research to date, few treatments are available to accelerate or improve recovery after facial nerve injury, and none prevents the adverse effects of aberrant regeneration and its clinical correlate, synkinesis.16

Our laboratory has recently adapted a rodent whisker movement monitoring system to quantitatively measure return of facial nerve function after injury.17-20 This system automatically measures the amplitude, velocity, and acceleration of whisks, providing a useful tool for precise quantification of the timing and completeness of facial nerve recovery after injury. We have found that transection-injured animals recover poorly, barely achieving measurable recovery after 4 months.18,19 This poor recovery is commensurate with the suboptimal clinical...
recovery seen in patients after a facial nerve transection and repair or电缆grafting. Our objectives in this study were to corroborate the recently reported improved functional recovery of whisking associated with daily mechanical whisker stimulation and to compare mechanical stimulation performed by daily whisker protraction with that performed by daily whisker retraction under the hypothesis that different directions of whisker stimulation would lead to different functional levels of recovery.

METHODS

PREPARATION FOR RIGID HEAD FIXATION

Forty female Wistar-Hannover rats (Charles River Laboratories, Wilmington, Massachusetts), weighing 200 to 250 g, were handled daily for 2 weeks prior to surgical manipulation to condition them to behavior testing. Subsequently, all rats underwent surgical insertion of a lightweight titanium head implant that provided a set of 4 external attachment points for rigid head fixation, as previously described. One week after head-fixation device implantation, the rats were conditioned to a body restraint apparatus by brief daily placements into a fitted sack. In the third week, head restraint was added to the daily conditioning regimen. After the third week, the rats were sufficiently conditioned to undergo head and body restraint without struggling or signs of stress, and presurgical baseline testing was performed. Rats were not food or water deprived prior to testing, and all experimentation was conducted under protocols approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee.

SURGICAL PROCEDURE

Rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg) (Fort Dodge Animal Health, Fort Dodge, Iowa) and medetomidine hydrochloride (0.3 mg/kg) (Orion Corporation, Espoo, Finland) in normal saline. The left infraauricular area was shaved and steriley prepared. Left facial nerve exposure involved a preauricular incision, reflection of the parotid gland, and visual identification of the main trunk of the facial nerve. The common trunk was electrically stimulated with a nerve stimulator (Montgomery Nerve Stimulator; Boston Medical Products, Westford, Massachusetts) at a setting of 1 mV to verify complete hemifacial movement. The nerve was then transected, and the cut ends were microsurgically reconnected with two 9-0 nylon sutures (Ethicon Inc, Somerville, New Jersey). The wound was then closed in a single layer with absorbable suture, and the anesthetic was reversed with a subcutaneous injection of 0.05 mg/kg of atipamezole hydrochloride. Rats were allowed to recover on a warming pad and were monitored postoperatively for signs of discomfort, including changes in grooming, social interaction, and maintenance of normal body weight.

MECHANICAL STIMULATION

All experimental animals received systematic mechanical stimulation to the whiskers 5 days per week, starting on the first postoperative day. Over a 5-minute period, with the animal held securely against the body of the handler to limit head movement, approximately 45 strokes per minute were delivered to the vibrissae in either a posterior-anterior direction (protraction group) or in an anterior-posterior direction (retraction group), using a soft-bristled paint brush (Figure 1). Care was taken to avoid stroking the whisker pad during stimulation. Animals rapidly habituated to the procedure and did not show signs of stress during or after manipulation.

FUNCTIONAL RECOVERY TESTING

Baseline whisking testing was performed preoperatively, and initial postsurgical testing was performed 1 week after facial nerve manipulation. Weekly postoperative testing of the animals continued across postoperative weeks 4 through 8 and was conducted a final time at week 15. Whisking recovery was monitored by our previously validated testing apparatus. Briefly, on the day of testing, the animals were placed in the body restraint device; right and left C-1 whiskers were marked using polyimide tubes (SWPT-045 and SWPT-008; Small Parts Inc, Logansport, Indiana) to increase their detectability; and then the rats were placed into the monitoring apparatus. The horizontal movement of the marked C-1 whiskers was independently tracked using commercial laser micrometers (Metralight, Santa Mateo, California) and a data-acquisition computer. A computer-controlled air valve was used to deliver 10-second sustained flows of scented air toward the snout to elicit whisking behavior at 2 random time points during each 5-minute data-recording session per animal.

DATA ANALYSIS

We detected and analyzed the 3 largest-amplitude whisks in automated fashion for each rat on each day of recording using software adapted from Bermejo et al. We normalized the data for each animal across the 2 sides of the face by dividing the whisking amplitude on the injured side by the amplitude on the uninjured side to determine the relative recovery.
amplitude was calculated. Independent, 2-sample, 1-tailed calculations were then repeated for velocity and acceleration. The top 3 amplitude values were normalized to the uninjured side and the average was calculated for the period of maximum postoperative weeks 4, 7, and 15. The top 3 amplitude values were statistically significant differences in relative velocity (protraction vs normative data: P = .03 at 4 weeks and P = .01 at 7 weeks, 1-tailed t test) and acceleration (protraction vs normative data, P < .001 at 7 weeks and P = .03 at 15 weeks, 1-tailed t test) between the protraction and retraction groups and our normative data at multiple time points (Table). At 7 weeks the protraction group demonstrated a significant improvement in acceleration compared with the retraction group (P < .001). For all other time points and kinematic measures the protraction group (n = 15-18) trended toward improved functional recovery compared with the retraction group (n = 18); however, no statistical significance was demonstrated between the experimental groups. (Table).

Examination of the top 3 performers in each group during postoperative weeks 4, 7, and 15 revealed a dramatic improvement in the protraction group compared with the retraction and control groups (Figure 3). One animal in the protraction group obtained nearly symmetric whisking by week 7 (Figure 4).

A plateau of recovery was achieved for whisking amplitude, velocity, and acceleration on the operated sides of all animals between postoperative weeks 7 and 15. On postoperative week 15, animals continued to exhibit a significant difference for amplitude, velocity, and acceleration between the operated and nonoperated sides (P < .005 for all, 1-tailed t test), indicating that complete recovery of whisking function was not achieved at the 15-week postoperative period for either mechanically stimulated group.

RESULTS

Rats were housed in groups of 2 to 3 animals per cage and demonstrated normal social, grooming, and feeding behavior during the 15 weeks of postoperative observation. There were no postoperative wound infections after either the head fixation device placement or the facial nerve transection procedure. There was an attrition rate of 20% of the rats across the 15-week postsurgical survival period due to head fixation device failure or generalized poor adaptation to the testing apparatus.

Two rats in the retraction group and 3 rats in the protraction group were not included at any time point due to early implant failure. Three additional rats in the protraction group were killed after postoperative weeks 4, 7, and 8 secondary to implant failure. Therefore, at the 15-week postsurgical time point there were 18 rats in the retraction group and 14 rats in the protraction group.

Preoperative testing revealed that protraction, retraction, and control animals demonstrated symmetrical release and left whisking, with relative amplitudes (SEs) of 1.0 (0.023), 1.0 (0.030), and 0.9 (0.191), respectively. Animals had complete absence of whisking function on postoperative week 1 and measurable whisking recovery by postoperative week 4 in both the protraction and retraction groups. This was significantly greater than the recovery observed without mechanical stimulation in our normative data set (unpublished data). There were statistically significant differences in relative amplitude between the protraction group and our normative data set (P = .01, P = .04, P = .01, P = .04, and P = .046 at weeks 4, 5, 6, 7, and 8, respectively; 1-tailed t test) and the retraction group and our normative data set (P = .005, P = .02, and P = .045 at weeks 4, 6, and 7, respectively; 1-tailed t test) at multiple time points (Figure 2). In addition, there were statistically significant differences in relative velocity (protraction vs normative data: P = .03 at 4 weeks and P = .01 at 7 weeks, 1-tailed t test) and acceleration (protraction vs normative data, P < .001 at 7 weeks and P = .03 at 15 weeks, 1-tailed t test) between the protraction and retraction groups and our normative data at multiple time points (Table). At 7 weeks the protraction group demonstrated a significant improvement in acceleration compared with the retraction group (P < .001). For all other time points and kinematic measures the protraction group (n = 15-18) trended toward improved functional recovery compared with the retraction group (n = 18); however, no statistical significance was demonstrated between the experimental groups. (Table).

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COMMENT

Facial nerve transection and epineural suture repair typically result in poor whisker function,14,19 even after 16 weeks of recovery or longer (unpublished data).18 Because the results of most surgical nerve repairs remain generally disappointing, the discovery by Angelov et al14 in 2007 that mechanical stimulation improves functional recovery of whisking compared with surgical repair of nerve transection alone was remarkable. The present study tried to verify that interesting finding, further quantify the whisking kinematics during the recovery process, and examine whether the particular nature of mechanical stimulation (protraction vs retraction) is important in enhancing functional recovery.

Recent evidence shows ipsilateral mechanical stimulation of the whisker pad and protraction of the vibrissae lead to recovery of normal whisking function,11 and...
manual stimulation of the orbicularis oculi muscle improves blink responses.\textsuperscript{14,23} Normal whisking motion is also recovered after facial nerve transection and repair in blind rat strains, where functional demands on whisking and whisker sensory feedback are heightened.\textsuperscript{15,24} In addition, mechanical stimulation under the mandible and physiotherapy, and the motor reanimation specialists because of the pressing need for more effective treatments for patients with facial paralysis.\textsuperscript{16} Neuromuscular retraining has been shown in a few small evidence-based clinical studies to improve facial function.\textsuperscript{27-29} However, there is lack of consensus regarding which manipulations and modes of sensory feedback are most effective. Current treatments include electromyographic and visual feedback,\textsuperscript{27,28} physiotherapy,\textsuperscript{29} and the motor rehabilitation method of Kabat\textsuperscript{28,31}; however, the underlying neural and physiologic mechanisms for improved functional outcomes are not known. This points to the importance of a quantitative animal model in which to study the mechanisms of improved recovery.

### Table. Relative Whisking Kinematic Data for the Transected Side\textsuperscript{a}

<table>
<thead>
<tr>
<th>Week</th>
<th>Amplitude</th>
<th>P Value</th>
<th>Velocity</th>
<th>P Value</th>
<th>Acceleration</th>
<th>P Value</th>
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<td></td>
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<tr>
<td>Protraction</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>0.307 (0.202)</td>
<td>.02\textsuperscript{b}</td>
<td>0.486 (0.314)</td>
<td>.03\textsuperscript{b}</td>
<td>0.545 (0.329)</td>
<td>.10</td>
</tr>
<tr>
<td>7</td>
<td>0.362 (0.278)</td>
<td>.04\textsuperscript{b}</td>
<td>0.517 (0.302)</td>
<td>.01\textsuperscript{b}</td>
<td>0.652 (0.316)</td>
<td>&lt;.001\textsuperscript{b}</td>
</tr>
<tr>
<td>15</td>
<td>0.341 (0.194)</td>
<td>.046\textsuperscript{b}</td>
<td>0.049 (0.210)</td>
<td>.35</td>
<td>0.524 (0.278)</td>
<td>.03\textsuperscript{b}</td>
</tr>
</tbody>
</table>

| Retraction | | | | |
| 4 | 0.258 (0.129) | .04\textsuperscript{b} | 0.400 (0.180) | .06 | 0.514 (0.212) | .09 |
| 7 | 0.246 (0.086) | .065\textsuperscript{b} | 0.394 (0.144) | .02\textsuperscript{b} | 0.302 (0.124) | .28 |
| 15 | 0.324 (0.314) | .13 | 0.508 (0.306) | .08 | 0.595 (0.381) | .01\textsuperscript{b} |

| Control | | | | |
| 4 | 0.187 (0.109) | NA | 0.313 (0.149) | NA | 0.422 (0.176) | NA |
| 7 | 0.194 (0.089) | NA | 0.292 (0.129) | NA | 0.329 (0.142) | NA |
| 15 | 0.231 (0.122) | NA | 0.378 (0.192) | NA | 0.348 (0.169) | NA |

Abbreviation: NA, not applicable.

\textsuperscript{a}Data are reported as a fraction of control side function (SD) unless otherwise indicated.

\textsuperscript{b}Significant improvement compared with the control group (1-tailed t test).

\textsuperscript{c}Significant improvement compared with the retraction group (2-tailed t test).

Our study was designed to reproduce and further elucidate the actions responsible for the positive effects of mechanical stimulation found by Angelov et al\textsuperscript{14} by altering the technique used to deliver mechanical stimulation. In the earlier study,\textsuperscript{14} the whiskers and whisker pad were simultaneously stimulated in the direction of whisker protrusion (with passive retraction) by finger massage. In the current report, we compared whisker protrusion with retraction and attempted to avoid direct mechanical stimulation of the whisker pad to isolate the stimulatory effects to the whiskers alone. We found that massage of the whisker pad along with the whiskers is not necessary for enhanced whisking recovery (compared with historical controls) and that whisker protrusion stimulation appears to produce better functional recovery than whisker retraction, although recovery was modest compared with that found by Angelov et al\textsuperscript{14}. However, when the top performers in each group were evaluated, we found dramatic functional improvement in the protrusion group compared with the control group, with 1 animal in the protrusion group regaining symmetric whisking function.

Several possible explanations exist for the improved functional outcome observed in this study. Both protrac-
tion and retraction of rodent vibrissae are under active muscular control. The nasalis initiates protraction, and the intrinsic muscles pivot the vibrissae further forward. Retraction involves relaxation of the nasalis and the intrinsic muscles and contraction of the caudal extrinsic muscles, nasolabialis, and maxillolabialis, pulling the vibrissae backward. Passive mechanical protraction of the vibrissae stretches the caudal extrinsic muscles and involves a large arch of motion. Retraction of the vibrissae stretches the intrinsic muscles but involves a smaller arch of motion. The fact that both retraction and protraction actions stretch whisking musculature could explain the benefit to recovery that both manipulations demonstrated. The protraction group may have trended toward improved recovery because of the greater degree of movement provided by passive mechanical protraction compared with that of retraction owing to the whiskers starting from a relatively retracted position at rest (between strokes). The improved recovery found by Angelov et al may represent an additional benefit of direct whisker pad manipulation, which possibly provides greater somatosensory input than does isolated whisker manipulation. In addition, neither daily handling nor an enhanced environment was shown to improve recovery unless daily whisker pad stimulation was also provided. These findings point toward the potential importance of both somatosensory input and proprioceptive input in recovery of the facial nerve function after injury.

In conclusion, the present study demonstrates improved functional recovery from daily mechanical retraction or protraction of whiskers after facial nerve transaction injury and repair in rats. These results are consistent with prior findings of enhanced facial nerve recovery after mechanical whisker protraction in rats and further indicate that the benefit of such stimulation may be derived from trigeminal nerve feedback. Future research will be directed toward better understanding the mechanisms of enhanced functional recovery brought about by mechanical stimulation so that similar interventions can be explored in human facial palsy.

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Author Contributions: Drs Lindsay and Hadlock had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Lindsay, Heaton, Smits, Vakharia, and Hadlock. Acquisition of data: Lindsay, Edwards, Smits, Vakharia, and Hadlock. Analysis and interpretation of data: Lindsay, Heaton, Edwards, Smits, Vakharia, and Hadlock. Drafting of the manuscript: Lindsay and Heaton. Critical revision of the manuscript for important intellectual content: Heaton, Edwards, Smits, Vakharia, and Hadlock. Statistical analysis: Lindsay, Heaton, Edwards, and Smits. Obtained funding: Hadlock. Administrative, technical, and material support: Vakharia and Hadlock. Study supervision: Hadlock.

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