Whisking Recovery After Automated Mechanical Stimulation During Facial Nerve Regeneration

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IMPORTANCE Recovery from facial nerve transection is typically poor, but daily mechanical stimulation of the face in rats has been reported to remarkably enhance functional recovery after facial nerve transection and suture repair. This phenomenon needs additional investigation because of its important clinical implications.

OBJECTIVE To determine whether automated mechanical stimulation of the whisker pad improves whisking recovery after facial nerve transection and repair in a rat model.

DESIGN AND SETTING Sixty-one rats underwent unilateral facial nerve transection and suture repair and were randomized into 8 groups. Six groups received daily automated whisker or whisker pad mechanical stimulation including 0.5-, 1.5-, and 8.0-Hz patterns. Two control groups received restraint without stimulation. Treatment started on postoperative day 8, occurred 5 days per week, and lasted throughout 15 weeks of recovery. Whisking amplitude, velocity, and acceleration were quantified weekly for 15 weeks.

INTERVENTIONS Unilateral facial nerve transection, suture repair, and, for 6 groups, daily automated whisker or whisker pad mechanical stimulation.

MAIN OUTCOMES AND MEASURES Quantification of whisking amplitude, velocity, and acceleration.

RESULTS Rats receiving the low frequencies of stimulation of the whiskers or whisker pad did not demonstrate enhanced whisking recovery, and rats receiving stimulation at 8.0 Hz showed significantly worse whisking recovery compared with controls and previously published groups receiving lower dose manual stimulation.

CONCLUSIONS AND RELEVANCE Although daily manual whisker pad stimulation has been shown to enhance whisking recovery, rats in this study did not demonstrate improved whisking recovery after automated mechanical stimulation across a wide range of driving frequencies. Moreover, faster stimulation (8.0 Hz) was actually detrimental to recovery. Further work is needed to understand the relationship between stimulation patterns and the physiologic mechanisms underlying improved or worsened functional outcomes after facial nerve transection and repair.

LEVEL OF EVIDENCE NA
Facial paralysis is a disorder with profound consequences, both functional and psychosocial. Causes of facial nerve paralysis are myriad, stemming from surgical, infectious, traumatic, congenital, and idiopathic factors. Among the various consequences, incomplete eye closure (leading to exposure keratopathy), external nasal valve obstruction, oral incompetence, speech and articulation problems, esthetic impairments, and the inability to express emotions through facial musculature are most clinically important. Treatment options comprise physical therapy, nerve transfers, muscle transfers, and static surgical techniques. The results, even following aggressive treatment, remain variable and often disappointing. After nerve repair, the slow rate of nerve regeneration can lead to degeneration of the motor end organ and permanent loss of function. In addition, axonal misrouting can develop, leading to synkinesis.

One driving question for facial nerve regeneration research groups is how to accelerate and improve facial nerve regeneration. The rat model is widely used to study interventions that affect speed and completeness of facial nerve recovery. The rat facial nerve is anatomically comparable to the human facial nerve, and recovery is highly quantifiable by measurement of whisking kinematics. Research has focused on pharmacologic, electrical, and mechanical interventions to accelerate and improve facial nerve regeneration; mechanical intervention has demonstrated the most promising results to date. The application of mechanical stimulation to the facial muscles during regeneration of the facial nerve could be relatively easy to administer and would be of great value in clinical settings where recovery is expected. However, more thorough exploration of the therapeutic potential of this treatment option is required, and the underlying physiologic mechanisms must be better understood before it can become part of routine clinical care of patients recovering from facial paralysis.

In previous studies from our laboratory and other laboratories, mechanical whisker and whisker pad stimulation has been delivered manually. Our laboratory recently developed a whisk assist (WA) system for delivering controlled and quantifiable patterns of mechanically driven whisking after rat facial nerve injury. This WA apparatus drives or assists whisker movement on the horizontal (dominant) plane of natural whisking, and is well tolerated by head-fixed (restrained) animals. In the present study, we examined the effects of several preprogrammed WA patterns during recovery from facial nerve transection and suture repair. We studied larger groups of rats of previously promising conditions and piloted additional new conditions under the hypothesis that WA treatment would enhance the speed and/or completeness of whisking recovery compared with none in control (CNTR) animals.

Methods

Conditioning and Head Fixation
Sixty-one female Wistar-Hannover rats (Charles River Laboratory) weighing 200 to 250 g each were handled on a daily basis 5 days per week for 1 week to acclimate them to human handling. All rats then underwent implantation of a titanium head fixation device, as previously described. The device has 4 lateral extensions that provide points of attachment with an external framework for head fixation. Two weeks after implantation of the head fixation device, the rats were progressively conditioned to a body and head restraint apparatus 5 days per week. When the animals were sufficiently conditioned to undergo head and body restraint without struggling or other signs of stress (typically after 2 weeks), unilateral facial nerve cut and suture repair surgery was performed. All experimentation was conducted under protocols approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee.

Facial Nerve Cut and Suture Repair Surgery
Rats were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) (Fort Dodge Animal Health) and dexmedetomidine hydrochloride (0.5 mg/kg) (Orion Corporation). The left facial nerve was exposed via a preauricular incision, and ipsilateral parotidectomy was performed. The main trunk and dominant branches of the facial nerve were identified. The main trunk of the nerve was sharply transected and microsurgically reconnected with two or three 10-0 epineural nylon sutures (Ethicon Inc). The wound was closed in a single layer with absorbable suture. All surgical procedures were performed by a single microsurgeon with substantial neurosurgery experience.

The anesthetic was reversed with a subcutaneous injection of atipamezole hydrochloride (0.05 mg/kg) (Orion Corporation). Postoperatively, the rats were monitored for signs of discomfort, weight maintenance, cage behavior, and wound issues.

Mechanical Stimulation With the WA System
The WA system has been previously described. Briefly, rats are placed in a body and head restraint half-pipe, which is then positioned in the apparatus. The WA system is designed to move the whiskers on one side of the face in the horizontal plane. The automated mechanical stimulation is delivered via a servomotor-controlled rod holding either a comb with 8 vertical tines that contact all of the prominent whiskers when the rod is moved horizontally or a brush that contacts the whisker pad for direct pad surface stimulation (Figure 1). The animals were randomized into 8 groups (Table). The most promising treatment patterns of Heaton et al were chosen to test in larger groups of rats; for 3 experimental groups, the mechanical stimulation treatment patterns moved the whiskers 60 to 70 degrees at a rate of 8.0 Hz, with these 3 groups differing in how many treatment sessions were delivered per day or whether the stimulation was continuous or intermittent during the treatment sessions. Three additional treatment patterns of the same amplitude were tested: 1 was whisker movement at 1.5 Hz, and for the other 2 groups, the WA apparatus was altered to provide both whisker and whisker pad stimulation at 0.5 Hz via the head of a soft-bristled toothbrush in place of the comb (Figure 1). These latter 3 low-frequency conditions were chosen to better emulate the manual mechan-
cal stimulation evaluated in prior studies. All treatment patterns started at postoperative day 8. Control animals were restrained for 20 minutes per day in an apparatus similar to the WA system, with the WA comb against the whisker pad, but without comb movement.

Functional Recovery Testing
Whisking function was assessed weekly throughout the 15-week recovery period using our laboratory’s previously validated testing apparatus. Briefly, the rats were placed in head and body restraint and positioned in the testing apparatus for 5 minutes of continuous recording per recording session. Movement of lightweight markers threaded onto a representative prominent whisker (row C, whisker 1) on the right and left was tracked by laser micrometers (MetrA Light) positioned adjacent to each whisker pad, and data on whisker movement were saved by custom data acquisition software.

Statistical Analysis
For each recording session, the 3 largest amplitude whisks on each side of the face were automatically identified and measured for amplitude, velocity, and acceleration using software adapted from Bermejo et al. Whisking function on the recovering side was analyzed in relationship to whisking on the healthy side to account for daily variation in whisking effort because whisking is typically symmetrical across both sides of the face. The recovery variables of whisking amplitude, acceleration, and velocity were each averaged by week across weeks 3 to 15 of recovery, and 1-way analysis of variance (ANOVA) was performed to test for overall treatment effects among the 8.0-Hz experimental and CNTR groups for each variable. Multiple post hoc Tukey tests were performed (as appropriate) after establishing main effects to determine which mechanical stimulation treatment groups differed from each other and from CNTR rats. These data were compared with previously published data, with 1-way ANOVA and Tukey post hoc tests. The small group sizes of the 3 low-frequency stimulation groups (Table) precluded meaningful statistical comparison, but data from these groups are presented descriptively with the other groups for comparison. Statistical testing was performed with SPSS software, version 16.0 (SPSS Inc).

Results
During the 15-week postoperative recovery period, 8 animals (13%) were excluded from the study because of head fixation device failure. This attrition rate was consistent with our laboratory’s prior findings using the head fixation device, and we have found this to be equal to or lower than attrition when simple head-mount screws are reinforced with adhesives over extended survival periods (eg, 15 weeks). The excluded animals were relatively equally spread across the groups (CNTR-A, 1 [2%]; CNTR-B, 2 [3%]; 8.0 Hz–A, 2 [3%]; 8.0 Hz–B, 1 [2%]; 8.0 Hz–C, 1 [2%]; 1.5 Hz, 0; 0.5 Hz–A, 0; and 0.5 Hz–B, 1 [2%]). There were no postsurgical wound infections after either the head fixation device implantation or the facial nerve transection and repair surgery, and all rats demonstrated normal feeding and social behavior.

Figure 2 shows the mean recovery of whisking amplitude across weeks 3 to 15 as a ratio of whisking amplitude for the nerve-repaired side divided by the healthy side. No trend of improvement was observed in animals undergoing these pilot WA conditions. Statistical analyses were performed with the 8.0-Hz experimental groups. Postoperative whisking amplitude data for the 8.0-Hz experimental and CNTR groups are

Figure 1. Rat in the Whisk Assist Apparatus
shown in Figure 3. A 1-way ANOVA demonstrated an overall statistically significant difference among these 5 experimental groups in relative whisking amplitude across weeks 3 to 15 ($P < .001$). Tukey post hoc analysis found statistically significant differences between CNTR-A and 8.0 Hz-A ($P = .02$), between CNTR-A and 8.0 Hz-C ($P < .001$), and between CNTR-B and 8.0 Hz-C ($P = .009$) (Figure 3B), with the WA groups performing more poorly. Results of whisking velocity and acceleration recovery were qualitatively and quantitatively similar to whisking amplitude recovery (data not shown).

Because there were no statistically significant differences between the CNTR groups and among the 8.0-Hz treatment conditions in the present study, the CNTR-A and CNTR-B groups were combined (all CNTR), as were the 8.0 Hz–A, Hz–B, and Hz–C groups (all 8.0 Hz) for further comparison with manual mechanical stimulation (MMS) data from our laboratory’s prior report.15 The mean recovery of whisking amplitude across weeks 3 to 15 in the MMS group was used for comparison. A statistically significant difference was found among these 3 groups ($P < .001$; 1-way ANOVA) (Figure 4), and Tukey post hoc analysis showed statistically significant differences between all 8.0 Hz and MMS ($P = .004$), and between all CNTR and all 8.0 Hz ($P = .001$), with CNTR animals and MMS animals performing better than WA animals. The mean recovery of whisking amplitude across weeks 3 to 15 for the MMS group from the prior report14 did not differ significantly from the all CNTR group of the present study ($P > .05$).

**Discussion**

Functional recovery from facial nerve transection and surgical repair is typically poor in rats and humans, providing an opportunity to test interventions intended to enhance facial nerve regeneration in rats that might ultimately translate to humans. Rat whisking movement begins to reappear approximately 3 weeks after unilateral facial nerve transection and repair, improves steadily for several weeks, and generally plateaus by 2 to 4 months at only approximately 25% of the whisking amplitude relative to the contralateral healthy side of the face.5,10–12,14 Previous studies10–14 have found enhanced functional recovery from brief, daily MMS of the whiskers and/or whisker pad delivered during recovery from unilateral facial nerve transection and repair. Such enhancement has ranged from a modest 10% improvement in relative whisking amplitude reported by our laboratory10,11 to complete (symmetrical) whisking recovery observed by others.12,14

We sought to deliver multiple patterns of whisker and whisker pad mechanical stimulation under greater experimental control than that in previous studies, with the goal of identifying optimal treatment patterns and potentially resolving discrepancies among prior outcomes. Based on pilot data,15 we anticipated that the 8.0-Hz stimulation patterns delivered in the present study would show greater enhancement of functional outcome compared with our prior moderate levels of stimulation.10,11,15 To the contrary, functional outcome after high-dose WA treatment was impaired relative to that in the CNTR animals, suggesting that high-dose WA treatment can cause deleterious overstimulation of the whisker pad. Moreover, lower-dose WA movement of the whiskers (1.5 Hz via comb interface) and/or whisker pad surface (0.5 Hz via brush interface) likewise failed to improve whisking recovery in the present study, drawing into question the cause of the complete recovery achieved after MMS performed in a different laboratory.12,14

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**Table. WA Treatment Programs**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>WA Pattern</th>
<th>Sessions/d</th>
<th>Total Time in Apparatus, min/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNTR-A</td>
<td>8</td>
<td>No stimulation</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>CNTR-B</td>
<td>8</td>
<td>No stimulation</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>8.0 Hz–A</td>
<td>16</td>
<td>Constant</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>8.0 Hz–B</td>
<td>8</td>
<td>Constant</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>8.0 Hz–C</td>
<td>8</td>
<td>5 s per 30 s</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>1.5 Hz</td>
<td>3</td>
<td>Constant</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>0.5 Hz–A*</td>
<td>5</td>
<td>Constant</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>0.5 Hz–B*</td>
<td>5</td>
<td>Constant</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

Abbreviations: CNTR, control; WA, whisk assist.
* These conditions included direct stimulation of the whisker pad surface.

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One possible mechanism underlying the apparent deleterious effects of high-dose (8.0-Hz) stimulation is fatigue of the facial mechanoreceptors during prolonged activation. The work of Pavlov et al indicated that intact sensory input is required for mechanical stimulation to provide a benefit and that mechanical stimulation delivered in the absence of normal whisker pad sensation (after infraorbital nerve cut) not only fails to enhance functional outcome but also worsens whisking recovery. Whisker pad sensation may have been diminished or interrupted during intensive mechanical stimulation in our study resulting from mechanoreceptor fatigue, thereby resembling the sensory nerve lesion effect reported by Pavlov et al and leading to reduced functional outcome. However, this does not explain why lower-frequency stimulation (0.5-1.5 Hz) failed to enhance whisking recovery and indicates that further experimentation is required to shed light on the interaction of sensory feedback and motor axon regeneration in the whisker pad.

An additional explanation for the lack of benefit from the 8.0-Hz WA stimulation is that direct mechanical contact with the whisker pad may be required to evoke a beneficial regenerative effect. A denervated muscle undergoes various changes depending on the delay before reinnervation, including loss of muscle mass, diminished blood circulation, shrinking of connective tissue, and adhesion (fibrosis). Mechanical stimulation of the whisker pad itself (instead of just whisker movement) may help to minimize these sequelae of denervation by maintaining whisker pad health while the facial nerve is regenerating and perhaps provide an optimal interaction between trigeminal and facial brainstem nuclei, as noted in the previous paragraph. In the initial version of our WA system, the oscillating comb contacted whiskers close to their exit from the pad (Figure 1A), but the comb had little contact with the pad itself. Given that the prior studies that found the greatest enhancement of facial nerve regeneration involved stimulation of both the whiskers and the pad through fingertip stroking of the pad, we modified the WA hardware for groups 0.5 Hz-A and 0.5 Hz-B in the present study to provide direct pad contact via a soft bristle brush pressing against the pad surface to emulate fingertip pressure (Figure 1C). However, this pad-stimulating condition failed to enhance whisking recovery, leaving us at a loss for why relatively low-frequency stimulation did not enhance regeneration as had been seen with manual stimulation in prior reports.

One potentially important difference between whisker pad stimulation delivered in the present study vs handheld delivery used in prior studies is the heightened stress that rats may have experienced under rigid restraint within the WA system. Rats were extensively conditioned to human handling and
placement in restraint in the weeks before nerve injury and repair followed by WA treatment, and they did not exhibit signs of heightened stress under restraint (eg, vocalizing or struggling) while the WA system delivered stimulation compared with the stationary comb control condition. However, it is possible that WA treatment produced occult stress that offset potentially beneficial effects of the treatment, explaining why whisking recovery that was similar to, or worse than, that of the restrained CNTR rats, as has been demonstrated in prior studies; van Meeteren et al24 demonstrated that chronic intermittent stress impaired nerve regeneration in a sciatic nerve model. The nerve regeneration process is controlled by neuroendocrine, immunologic, and autonomic nervous system factors. Chronic stress deteriorates the efficiency of these processes; for example, activation of the autonomic nervous system causes epineural vasoconstriction and reduces endoneural nerve blood flow.24 Likewise, Amako and Nemoto25 found suppressed sciatic nerve recovery after water-immersion stress in rats.

Variability of the whisking amplitude within groups (Figure 3) and the fluctuation in mean whisking kinematics across all groups at most recovery time points is consistent with our prior observations and represents an inherent weakness in using this functional recovery measure. Because rats whisk with variable amplitude based both on muscle strength and intactness of innervation, as well as on mood, state of curiosity, arousal, and interest in the surrounding environment, among other factors, the assay itself may be relatively insensitive to small but real influences and interventions.

Further investigations of mechanical stimulation effects on nerve regeneration will require exploring ways to mitigate the stress associated with restraint during treatment delivery, such as sedation or delivery of appetitive rewards.

The effects of mechanical stimulation have been studied in other animal models of peripheral nerve regeneration,26–31 sometimes with conflicting results. For example, van Meeteren et al27 demonstrated that mild daily exercise (4 hours of hindpaw stretching) augmented functional recovery in the early phase (persisting into the late phase) after sciatic nerve crush in the rats, whereas Herbison et al22–23 showed that intense swimming did not enhance the repair of reinervated muscle and that treadmill running led to a deleterious effect on muscle function recovery.

Potential explanations for these inconsistencies include variations in the type of nerve injury, whether or not the nerve contains sensory axons,30 the type of mechanical stimulation delivered, and the duration and intensity of the stimulation.34 There are likewise conflicting results in the literature40–44 as described above with specific regard to the effect of mechanical stimulation on regeneration of the facial nerve. It is possible that, in our hands or apparatus, mechanical stimulation has not led to true enhancement in whisking recovery when the performance of CNTR and experimental groups is considered across our studies. The relative recovery of whisking amplitude for regenerated nerves vs the contralateral (healthy) side within rats has been approximately 10% better (on average, within studies) compared with that in simultaneous nerve-repaired CNTRs10,15 or historical CNTRs.11 However, had the present nerve-repaired CNTR group served as the point of comparison in our laboratory’s prior studies, then none of the previous stimulated groups would have shown an enhancement. This is illustrated by comparing the greatest prior enhancement of MMS11 with the present all CNTR group (Figure 4), yielding similar whisking amplitude after 15 to 16 weeks of recovery. This suggests that either daily restraint for 20 minutes (with a stationary whisker comb) provides the same degree of recovery enhancement as mechanical stimulation in all of the manual and automated forms our laboratory has tested to date,10,11,15 or that the effect of mechanical stimulation by our group has been negligible. Either way, we have repeatedly failed to replicate the complete symmetrical recovery caused by MMS of the whisker pad as previously reported.12–14 Differences in stimulation delivery techniques or whisking quantification methods across laboratories may contribute to these disparate findings,35 but our studies have shown that enhancing nerve regeneration through whisker pad manipulation is difficult to achieve at best and potentially detrimental at worst. Our failure to demonstrate recovery benefit despite our exhaustive use of myriad regimens of automated WA under systematic, highly controlled circumstances, with and without direct whisker pad stimulation, leads us to conclude that the benefit originally proposed may not represent a true, reproducible phenomenon.

Conclusions

Recovery of horizontal whisking was monitored for 15 weeks after unilateral facial nerve transection and repair in 61 rats, 45 of which received daily, automated WA mechanical therapy and 16 of which served as sham-stimulated CNTRs. Automated mechanical stimulation failed to enhance whisking recovery for low-frequency stimulation (0.5 Hz or 1.5 Hz) resembling the manually delivered patterns used in prior reports, and higher-frequency stimulation (8.0 Hz) resulted in worse recovery than that observed in CNTRs. Moreover, the CNTR group in the present study performed as well as the experimental groups in our laboratory’s prior reports, in which recovery was believed to have been enhanced by MMS (via whisker pad stroking with a fingertip10 or paintbrush11), drawing into question whether mechanical stimulation actually led to meaningful enhancement of whisking in those reports. The discrepancy between the modest or nonexistent whisking recovery enhancement caused by mechanical stimulation in some studies10,11,15 compared with the complete recovery reported by other groups12–14 requires further clarification given the potential clinical importance of this physical therapy intervention.
responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Kleiss, Knox, Hadlock, Heaton.

Acquisition of data: Kleiss, Knox, Malo, Hadlock.

Analysis and interpretation of data: Kleiss, Malo, Marres, Hadlock, Heaton.

Drafting of the manuscript: Kleiss, Malo, Hadlock.

Critical revision of the manuscript for important intellectual content: Kleiss, Knox, Marres, Hadlock, Heaton.

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Correction: This article was corrected on March 6, 2014, to fix the Abstract.

REFERENCES


