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Cable Grafting Permits Recovery Equivalent to Primary Repair in a Rat Facial Nerve

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Abstract

Objective—To compare functional recovery after cable grafting to recovery after primary repair in a rodent facial nerve model.

Study Design—Prospective, randomized, controlled animal study.

Methods—Sixteen female Wistar Hannover rats were divided into 2 groups: a control group (facial nerve transection and primary repair), and an experimental group (reversed autograft reconstruction of a 2cm neural gap). Whisker excursion was measured weekly for 70 postoperative days using laser micrometers.

Results—The control group exhibited the most rapid recovery, with significant return of whisker movement occurring during the 3rd postoperative week. The experimental group demonstrated return of function beginning in the 4th postoperative week, eventually achieving the same degree of function as the control group by the 6th postoperative week ($p = 0.68$).

Conclusions—Recovery of facial function after cable grafting appears to be slower than, but eventually equivalent to, recovery after primary neurotaphy in a rodent model. In this study we have established a benchmark for recovery of whisker movement across a 2cm rodent facial nerve gap, which will be used for comparison of different facial nerve gap bridging materials in future studies.

INTRODUCTION

The gold standard for facial nerve reconstruction after transection is microsurgical neurotaphy; coaptation of the divided nerve ends may be accomplished via different modalities, but primary repair is the technique of choice.¹ When a significant length of nerve has been lost and primary repair is no longer a viable option, interposition, or “cable” grafting, is generally considered to be the next rung on the reconstructive ladder.¹

To date, there have been no papers comparing recovery after interposition autografting to recovery after primary neurotaphy in the rat facial nerve. We sought to investigate recovery of facial function in the rat using a validated and highly quantitative method.^{2,3} Whisker excursion, or “whisking,” is the most readily measurable facial movement in the rat, and is produced by the combined action of extrinsic whisker pad muscles and intrinsic “sling” muscles attached to each of the approximately 25 dynamically controlled vibrissae within each pad.^{4,5} Whisker pad muscles are innervated by the buccal and marginal mandibular branches of the facial nerve,⁴ with either branch capable of supporting dynamic whisking.^{6,7} The present study was designed to quantify recovery after cable grafting of the buccal and marginal mandibular branches with respect to recovery after primary neurotaphy, both in order to make direct comparisons and to establish a functional baseline for recovery across a long neural gap for future whisker movement recovery studies.

MATERIALS AND METHODS

Sixteen female Wistar Hannover rats (Charles River Laboratories, Wilmington, MA) 90 to 105 days old and weighing 200 to 250g were used for the study under a protocol approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee, and the NIH guidelines for animal care and use were followed at all times. Eight animals were randomized to the experimental group, and 8 served as controls. All surgical procedures were performed under general anesthesia, induced with intramuscular ketamine (50mg/kg) (Fort Dodge Animal Health, Fort Dodge, IA) and medetomidine (0.5mg/kg) (Orion Corporation, Espoo, Finland).

Head Fixation and Conditioning

The preoperative animal conditioning protocol established by Hadlock et al in 2007 was followed.⁸ Animals were handled individually for 5 minutes daily over the course of one week to acclimate them to manipulation by humans. Then, titanium head fixation implants (Whitman Tool and Die, Whitman, MA) were placed, using 1.3 × 4mm titanium screws (Synthes CMF, West Chester, PA).⁸ Animals were allowed to recover from surgery for 2 weeks before proceeding with conditioning to the testing apparatus. Conditioning lasted between 3 and 4 weeks, until animals easily tolerated placement in the apparatus. Rats then underwent facial nerve manipulation as described below.

Surgical Procedure

Experimental Group—A preauricular incision was made on the left side of the face and carried down to the parotid gland, which was then removed in order to expose the underlying buccal branch of the facial nerve. This branch was followed in a retrograde fashion toward the pes anserinus, permitting identification of the main trunk, from which the marginal mandibular branch was identified. A transverse facial incision was then made from the base of the auricle to the lateral aspect of the whisker pad in order to expose the buccal and marginal mandibular branches all the way to the distal convergence of facial nerve branches, as described by Henstrom et al in 2012;⁶ any collateral rami encountered were divided. The nerve was then transected at the pes anserinus and at the distal convergence,

such that the buccal and marginal branches were resected en bloc, remaining joined both distally and proximally, resulting a single, 2 fascicle neural autograft, measuring 20 mm, ± 1 mm. The orientation of this conduit was then reversed, relocating the distal end proximally, and vice versa. Proximal and distal neurorrhaphies were performed using between 3 and 6 interrupted 10-0 nylon sutures (Ethilon, Ethicon, Somerville, NJ), taking care to ensure complete approximation of the epineurium around the circumference (Figure 1). The incisions were closed with running 3-0 polyglactin sutures (Vicryl, Ethicon, Somerville, NJ).

Control Group—The main trunk of the facial nerve was identified via a left preauricular incision, as described above, then transected sharply. It was immediately repaired with 2 to 4 interrupted 10-0 nylon sutures, taking care to reapproximate the epineurium around the entire circumference of the neurorrhaphy (Figure 1). The incision was then closed with a running polyglactin suture.

Functional Testing and Data Analysis

Whisking kinematic data were collected during weekly testing sessions for 10 consecutive weeks, following the protocol established by Heaton et al in 2008.² Briefly, while each animal was restrained in a body harness and head fixation apparatus, laser micrometers (MetraLight, San Mateo, CA) were used to measure whisking movements over the course of 5 minutes, on both the operated and unoperated sides (Figure 2). Polyimide tubes (SWPT-045 and SWPT-008, Small Parts, Logansport, IN) were used to facilitate micrometer tracking by visually enhancing the C-1 whisker.

During each testing session, software developed by Bermejo et al in 2004 was used to determine the amplitude of the C-1 whisker movements.⁹ The amplitudes of the 3 greatest whisker excursions in each testing session were then averaged by arithmetic mean. A one way ANOVA test with Bonferroni post hoc analysis was performed using the compressed data from postoperative days 21 through 70; there was negligible whisker movement in the first 3 weeks after facial nerve manipulation; a p value < 0.05 was considered to be significant. Data are reported as absolute amplitude values as well as “relative recovery,” or the ratio of the operated side whisking amplitude to the unoperated side, in order to minimize amplitude variations caused by daily inconsistencies in whisking effort.

RESULTS

All 16 animals had documented normal whisking movements prior to surgery, subsequently demonstrating immediate postoperative loss of whisking on the operated side of the face with preservation of movement on the unoperated side. Whisking amplitudes of 10° or less may arise from jaw movements or slight changes in head position, and are not included in further analysis. Average whisking amplitude did not increase above this potential noise level until the third postoperative week for the control group, and the fourth week for the experimental group (Figure 3). By postoperative day 42, recovery in the experimental group matched that of the controls; thereafter, the 2 groups remained closely associated and did not statistically differ ($p = 0.68$).

There were no surgical complications, although 4 animals were eliminated from the study due to failure of the head fixation implants: 2 from the experimental group and 2 from the control group. This was consistent with historical rates of head fixation device failure.

DISCUSSION

The results of this study indicate that autogenous cable grafting in rats produces functional recovery that is comparable, though slightly delayed, compared with that seen after primary repair of the facial nerve. Although the experimental and control groups demonstrated no statistically significant difference in recovery when assessed as a whole, there appears to be a trend toward more rapid initial recovery in the primary repair group relative to the cable graft group. The primary repair animals underwent main trunk transection and neuroorrhaphy with the orientation of the distal aspect of the main trunk maintained relative to the proximal segment; this would have resulted in the complete disruption of all neural elements, but with possible preservation of some internal orientation. Preservation of neural orientation was higher in these animals than in the experimental animals, who underwent cable grafting. Not only does a cable graft introduce a second neuroorrhaphy, but it is impossible to maintain alignment of neural elements since the internal architecture of the interposed nerve segment does not match that of the native nerve stump. This lack of alignment may have contributed to delayed recovery. Because the recovery of whisking function was brisk in both the experimental and control groups, and because the groups were comparatively small, it is not possible to conclude with certainty that there exists a lack of statistically significant difference in functional recovery between the groups.

Cable graft repair of long motor nerve defects remains problematic. Extensive research has been performed to identify a substitute for primary neuroorrhaphy in cases of nerve gaps long enough to preclude the possibility of tensionless primary repair. Thus far, no material has demonstrated a greater potential to promote functional recovery than an autogenous cable graft. Numerous biomaterials have been previously investigated for their ability to conduct and promote axonal regeneration, including type I collagen tubules,¹⁰ porous and smooth-walled poly-L-lactic acid, polylactic-co-glycolic acid,¹¹ and poly(glycerol sebacate) tubules;¹² vein grafts and small intestinal submucosa seeded with Schwann cells have also been studied.¹³ While histological and electrophysiological analyses of these materials often demonstrate fiber counts and compound action potentials approximating those encountered after repair via autologous cable graft, functional testing, when performed, provides uniformly poor results compared to neural autograft. Commercial synthetic neural conduits, such as copolyester poly(DL-lactide-epsilon-caprolactone) (Neurolac, Polyganics BV, Groningen, Netherlands), are available as well, but clinical results remain inconsistent at best.¹⁴ In addition to neural conduit materials, cytokines, such as vascular endothelial growth factor, and mesenchymal stem cells are often employed to augment neural regeneration, but even when combined with novel conduit materials, results from these are consistently surpassed by cable graft repair.^{10,13,14,15}

In the current study, we have employed a highly quantitative functional outcome measure in the rodent facial nerve cable graft model, leaving our group well-poised to investigate future alternatives to neural autograft for bridging long nerve gaps. Previously, quantification of

whisker movement recovery after cable grafting across a long neural gap was difficult to perform, but advances in surgical technique, data gathering technology, and experience over the last decade have provided an optimal platform for this research. Across a 10 year experience with rodent facial nerve manipulations, we have demonstrated consistency among our transection and repair control group whisker movement results.¹⁶ The natural history of rodent facial nerve recovery is well-documented and well-understood, and constitutes a dependable control set for comparison with cable autograft repair of the 20mm facial nerve gap utilized in our study. In turn, the data presented here, building on the primary neurotaphy benchmark, comprise a reliable standard against which to compare results from future neural reconstruction studies.

CONCLUSION

Herein we have demonstrated that cable grafting in the rat facial nerve model permits useful neural regeneration and partial recovery of function that is not statistically different from the results obtained after primary epineurial repair, across 10 weeks of convalescence. Our quantification of whisker movement recovery after cable grafting in the rat will serve as a baseline for future studies on rodent facial nerve regeneration, particularly in the areas of neural conduit and tissue engineering research. Additionally, difference between functional recovery after sensory and motor nerve cable grafting in the rodent facial nerve model is currently under investigation by our group.

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Fig 1.

Rat facial nerve anatomy with outlined locations and corresponding surgical photos showing representative nerve manipulations. Lower left: Facial nerve main trunk after transection and primary suture repair just proximal to the pes anserinus. Blue surgical drape material was placed behind the nerve to enhance visual contrast. Lower right: Neurorrhaphy of the reversed cable graft at the distal convergence, with the buccal and marginal mandibular branches shown running immediately adjacent to each other.

Fig 2.
Measurement of whisking function using laser micrometers.

Fig 3.

A) Recovery of function as represented by amplitude of whisker excursion. **B)** Relative recovery as represented by the ratio of whisker movement amplitude on the operated side to the unoperated side (a value of 1 would represent symmetrical whisking amplitude).