The gold standard for facial nerve reconstruction after transection is microsurgical neurorrhaphy; coaptation of the divided nerve ends may be accomplished by means of different modalities, but primary repair is the technique of choice.1 When a substantial 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 our knowledge, there have been no articles comparing recovery after interposition autografting with recovery after primary neurorrhaphy in a rodent model. 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 primarily 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 neurorrhaphy in order to establish a functional baseline for recovery across a long neural gap for future whisker movement recovery studies.
Methods

Sixteen female Wistar Hannover rats (Charles River Laboratories) 90 to 105 days old and weighing 200 to 250 g were used for the study under a protocol approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee; National Institutes of Health 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 hydrochloride (50 mg/kg) (Fort Dodge Animal Health) and medetomidine hydrochloride (0.5 mg/kg) (Orion Corporation).

Head Fixation and Conditioning

The preoperative animal conditioning protocol established by Hadlock et al8 in 2007 was followed. Animals were handled individually for 5 minutes daily over the course of 1 week to acclimate them to manipulation by humans. Then, titanium head fixation implants (Whitman Tool and Die) were placed, using 1.3 × 4-mm titanium screws (Synthes CMF). Animals were allowed to recover from surgery for 2 weeks before proceeding with conditioning to the testing apparatus. The conditioning period lasted between 3 and 4 weeks, until animals easily tolerated placement in the apparatus. Rats then underwent facial nerve manipulation.

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 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 to expose the buccal and marginal mandibular branches all the way to the distal convergence of facial nerve branches, as described by Henstrom et al6 in 2012; 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 in a single, 2-fascicle neural autograft, measuring 20 ± 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), taking care to ensure complete approximation of the epineurium around the circumference (Figure 1). The incisions were closed with running 3-0 polyglactin sutures.

Control Group

The main trunk of the facial nerve was identified via a left preauricular incision, as for the experimental group, then transected sharply. It was immediately repaired with 2 to 4 interrupted 10-0 nylon sutures, with care taken 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 al2 in 2008. Briefly, while each animal was restrained in a body harness and head fixation apparatus, laser micrometers (MetraLight) were used to measure whisking movements over the course of 5 minutes, on both the operated and unoperated sides (Figure 2). Polymide tubes (SWPT-045 and SWPT-008, Small Parts) were used to facilitate micrometer tracking by visually enhancing the C-1 whisker. During each testing session, software developed by Bermejo et al9 in 2004 was used to determine the amplitude of the C-1 whisker movements. The amplitudes of the 3 greatest whisker excursions in each testing session were then averaged by arithmetic mean. A 1-way analysis of variance 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...
nervemanipulation; \( P < .05 \) was considered significant. Data are reported as absolute amplitude values, as well as “relative recovery,” or the ratio of the operated side whisking amplitude to that of the unoperated side, to minimize amplitude variations caused by daily inconsistencies in whisking effort.

Results

All 16 animals had documented normal whisking movements prior to surgery and subsequently demonstrated 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. Mean 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 (\( P = .68 \)).

There were no surgical complications, although 4 animals were eliminated from the study because of 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 comparable to that seen after primary repair of the facial nerve, up to 10 weeks after nerve injury and suture repair. Although the experimental and control groups did not demonstrate a statistically significant difference in recovery when assessed as a whole, there seemed to be a trend toward more rapid initial recovery in the primary repair group relative to the cable graft group. However, 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 rule out a difference in functional recovery between the groups. In fact,
although such a difference is likely, we were unable to measure it using the current paradigm. Importantly, the relatively incomplete recovery of whisking using either cable grafting or primary neurorrhaphy affords investigators the opportunity to improve on currently achievable results via innovative neural repair methods and tissue engineering strategies.

One difference in cable grafting vs primary repair that may affect the timing and/or ultimate functional outcome after neurorrhaphy is the relative morphological characteristics of the cut and coapted nerve ends. With both nerve repair techniques, there is complete disruption of all neural elements, yet the epineurial and fascicular profiles can be expected to better match for primary repair if the nerve's anatomical orientation can be maintained through the transection and neurorrhaphy procedure. An autogenous cable graft, in contrast, not only introduces a second neurorrhaphy but may also present an anatomical mismatch for the coapted nerve ends that can impede regrowing axons (when they face nonneural tissues) or cause them to fail to enter the graft if the coapted ends do not match in size. The 2 neurorrhaphy locations and potentially imperfect anatomical alignment may have contributed to the apparent delay in initial recovery observed after autogenous cable grafting vs primary repair in the present study. Cable graft repair of long motor nerve defects remains problematic. Extensive research has been performed to identify a substitute for primary neurorrhaphy 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, porous and smooth-walled poly-L-lactic acid, poly lactic–co-glycolic acid, and poly (glycerol sebacate) tubules; vein grafts and small-intestinal submucosa seeded with Schwann cells have also been studied. Whereas 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 with neural autograft. Commercial synthetic neural conduits, such as copolyester poly(DL-lactide-ε-caprolactone) (Neurolac, Polyganics BV), are available as well, but clinical results remain inconsistent. In addition to neural conduit materials, cytokines, such as vascular endothelial growth factor, and mesenchymal stem cells are often used to augment neural regeneration, but even when combined with novel conduit materials, results from these are consistently surpassed by those of cable graft repair.

In the present study, we used a highly quantitative functional outcome measure in the rodent facial nerve cable graft model, laying the groundwork for future investigations of 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 past decade have provided an optimal platform for this research. The anticipated progression 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 20-mm facial nerve gap between the main trunk and distal convergence of the buccal and marginal-mandibular branches of the rat facial nerve. In turn, the data presented here, building on the primary neurorrhaphy benchmark, constitute a reliable standard against which to compare results from future neural reconstruction studies.

Conclusions

We have demonstrated herein that cable grafting in the rat facial nerve model permits useful neural regeneration and partial recovery of function when studied over a period of 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. In addition, the difference in functional recovery after sensory and motor nerve cable grafting in the rodent facial nerve model is currently under investigation by our group.

REFERENCES


