

# Baiting the Cross-Face Nerve Graft With Temporary Hypoglossal Hookup

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**Background:** Cross-face nerve grafting yields inconsistent neural regeneration, and methods that promote more robust axonal traversing of the graft would expand the indications for this procedure.

**Objective:** To test the hypothesis that hooking a cross-face nerve graft distally to a source of denervated muscle, rather than leaving it in the subcutaneous space, would positively affect neural ingrowth across the graft, based on elaboration of neurotrophins from the musculature.

**Methods:** Twenty-four rats underwent cross-face nerve grafting in which the right facial nerve buccal branch was transected and coapted to the graft. The graft was placed across the neck and into the left side of the face. The distal end of the graft was placed either in the left subcutaneous space, coapted to the marginal mandibular branch of the left facial nerve, or coapted to the distal stump of the transected left hypoglossal nerve. Eight control animals underwent right buccal branch transection and placement of a cross-face nerve graft without any proximal and distal hookup. After 12 weeks, all experimental groups

underwent hookup of the distal nerve graft to the left facial nerve buccal branch. Vibrissal function was assessed during the ensuing 12 weeks, and then the graft was harvested for histomorphometric analysis.

**Results:** After 12 weeks, there was a significant difference in axon counts between the group coapted distally to the tongue (hypoglossal hookup) and that coapted to the facial musculature (marginal hookup). Twelve weeks later, after distal cross-face nerve graft hookup, this difference was not statistically significant, although the hypoglossally baited group demonstrated statistically significantly greater fiber maturity. Recovery of vibrissal movement did not differ among treatment groups.

**Conclusion:** Baiting the cross-face nerve graft via temporary hookup to the distal hypoglossal nerve and tongue musculature appears to improve nerve ingrowth through a nerve graft across the face, although a corresponding improvement in facial muscle function was not observed.

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**A** WELL-ACCEPTED APPROACH to reanimation of the chronically paralyzed face is to perform a first-stage cross-face nerve graft using the sural nerve. This is followed 6 to 12 months later by transfer of vascularized muscle into the affected side and hookup of the cross-face graft to the nerve stump of the transferred muscle. Originally described by Harii et al,<sup>1</sup> this method is superior to other methods of facial reanimation because of its unique potential for restoring mimetic spontaneous smiling. While some surgeons report good success with this technique,<sup>2-4</sup> the results are inconsistent, even in experienced hands. Failures are unlikely to be related to vascular compromise because success using free muscle transfer powered by the ipsilateral masseteric branch of the trigeminal nerve is remarkably high.<sup>5</sup> There-

fore, the leading cause of failure is suspected to be poor traversing of motor axons from the contralateral facial nerve branches into, across, and eventually out of the sural nerve graft.

Major improvements in nerve coaptation have occurred over the past half century. Improved microsurgical magnification, instrumentation, and suture technique have contributed to this change.<sup>6</sup>

It is well recognized that denervated muscle itself provides a strong neurotrophic influence on a severed nerve. Classic experiments in which the proximal end of a nerve is inserted into a Y-shaped conduit and the fibers are given a "choice" between limbs of the Y, where one limb is hooked to the distal nerve and muscle and the other is left in the soft tissue, demonstrate that fibers tend to pursue the tract coapted to the denervated target. Similar experiments involving straight conduits

with different distal elements show the same results.<sup>7-9</sup> Based on these findings, factors elaborated by the distal nerve stump and muscle have been identified that promote axonal extension, and attempts to deliver these substances effectively to maximize their beneficial effect are ongoing.

In the present study, we sought to discover whether there was a significant increase in the number of axons traversing the cross-face nerve graft when the distal stump was coapted to the distal stump of a freshly transected motor nerve. We compared the findings of 3 experimental scenarios: (1) coaptation to a nerve innervating a large muscle mass (the tongue); (2) coaptation to a nerve innervating a smaller-volume muscle (the marginal distribution portion of the facial musculature); and (3) no distal neural coaptation. While the hypoglossal nerve may not be an appropriate target for human use for promoting cross-face sprouting in any clinical situation, the model was chosen based on the accessibility for coaptation and the knowledge that hypoglossal transection in the rat is well tolerated.

## METHODS

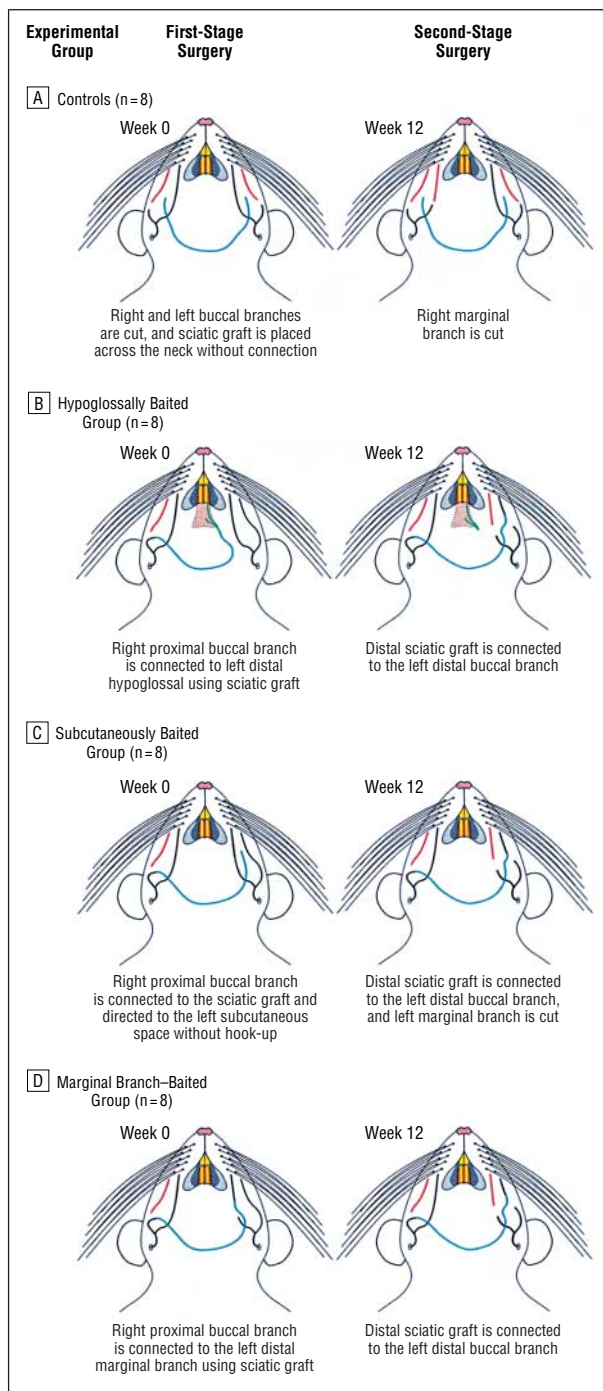
### SURGICAL PROCEDURE

Twenty-four adult male Fischer rats underwent cross-face nerve grafting. Institutional guidelines regarding animal experimentation were strictly followed. Each animal was anesthetized with combination ketamine/medetomidine, and the face, neck, and left thigh were shaved and sterilely prepared. The entire left sciatic nerve was harvested from close to the spinal cord to the distal lower extremity, yielding an approximately 6-cm nerve graft. The graft was placed in saline, and an apron incision from auricle to auricle was made in the neck. Both parotid glands, facial nerves, and the superficial neck structures were exposed. The right buccal branch of the facial nerve was identified and sharply transected, and a 1-cm segment of the distal stump was resected to leave a gap to inhibit spontaneous regeneration of fibers into the remaining stump.

The nerve graft was then brought into the neck field, reversed, and coapted to the proximal stump of the buccal branch using two or three 10-0 nylon sutures. The graft was draped across the neck under the apron flap at the level of the submandibular glands, and the distal end of the sciatic graft was treated 1 of 3 ways ( $n=8$  in each group) (Figure 1). In the first group, the graft was allowed to lie in the subcutaneous space. In the second group, the left marginal mandibular nerve was sharply transected, and the distal stump leading to its target facial musculature was coapted to the sciatic graft. In the third group, the hypoglossal nerve was exposed, sharply transected as a single-nerve trunk at the level of the anterior belly of the digastric muscle, and the distal stump was coapted to the sciatic graft.

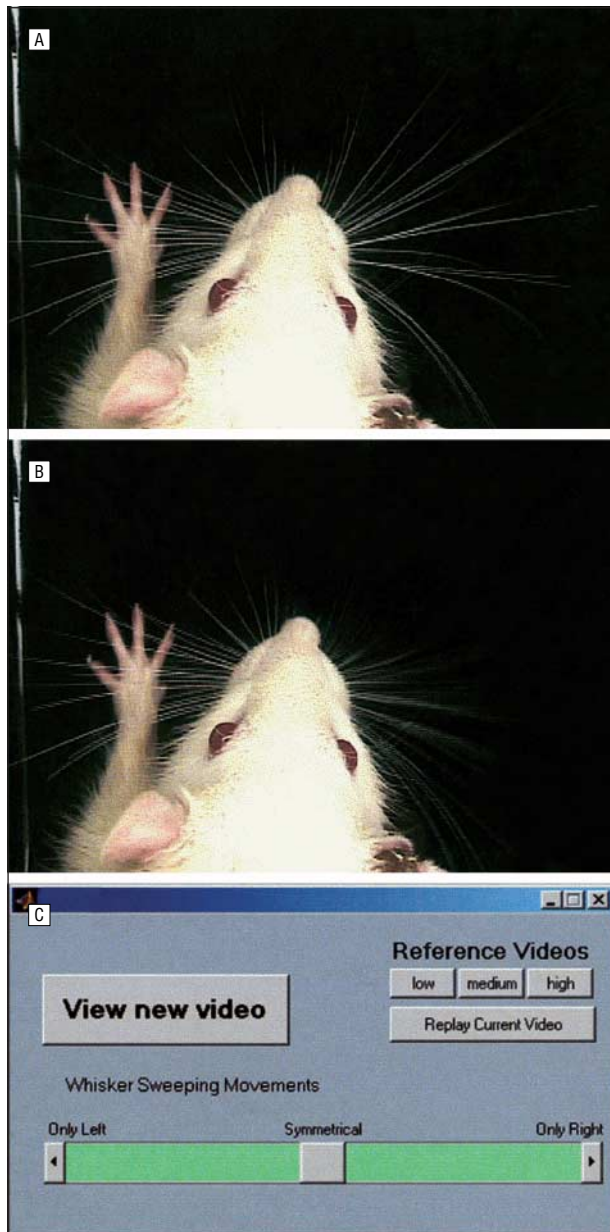
In a control group of 8 animals, both buccal branches were divided as described for the experimental groups, and the sciatic nerve was draped into the neck with no hookup at either end. All skin incisions were closed with polyglactin 910 (Vicryl 4-0 suture; Ethicon Inc, Somerville, NJ). Animals were administered an anesthesia-reversing agent, atipamezole (0.1 mg/kg), at completion of surgery.

After 12 weeks, the animals underwent second-stage surgery. For the 3 experimental groups, this involved exposure of the left side of the neck and face and identification of the sciatic graft and the left facial nerve. A biopsy specimen of the tip



**Figure 1.** Schematic illustration of our operative procedure: A, the control group with no proximal or distal hookup at either stage; B, the hypoglossally baited hookup and second-stage left buccal hookup; C, the subcutaneously baited group and second-stage left buccal hookup; and D, the marginal-mandibular-nerve-baited group and second-stage left buccal hookup. Cross-face grafts are shown in blue, transected distal facial nerve branches in red, and intact or reinnervated facial nerve in black. Hypoglossal nerve is depicted in green.

of the sciatic graft in the left side of the face or neck was fixed in 3% electron microscopy-grade glutaraldehyde for histomorphometric analysis. For the 2 groups with grafts coapted to recipient baiting nerves, the entire coaptation area, with several millimeters on either side of the suture line, was sharply excised and discarded. The left buccal branch of the facial nerve was then sharply transected, and a neurorrhaphy



**Figure 2.** Two video frames show the asymmetry in vibrissal movements soon after nerve transfer (A and B). A, The left vibrissae remain conspicuously stationary, while the right ones sweep forward during searching behavior. B, Video blurring is apparent in the right vibrissae during their normal rapid movement. C, A software interface provided the rater with a continuous sliding scale on which the midpoint represents symmetry in right/left vibrissal movement and the left and right extremes represent left and right movement only, respectively.

was performed between the cross-face nerve graft and the distal stump of the left buccal branch (Figure 1). To permit functional analysis of the left facial nerve function, the animals that had not undergone left marginal mandibular branch transection underwent it at this stage to completely denervate the vibrissal pad on the left. Thus, any returning left-sided vibrissal function could be assumed to be coming from regeneration through the cross-face nerve graft into the left buccal branch. Control animals with cross-face nerve grafts draped into the neck with neither proximal nor distal hookup underwent only harvest of a segment of the nerve graft and marginal mandibular branch transection for further hemifacial denervation.

The animals were allowed to live an additional 12 weeks after second-stage surgery to permit ingrowth from the cross-face graft into the left facial musculature. They were then killed, and the distal cross-face nerve graft and distal buccal branch were harvested for histomorphometric analysis.

## FUNCTIONAL TESTING

Immediately before and at 1 and 12 weeks after the second-stage surgical procedure, animals underwent video analysis to monitor vibrissal function (**Figure 2**). The degree to which vibrissal movement was symmetrical for the right and left sides of the face was used as a measure of functional innervation through the cross-face nerve graft. The vibrissae of all animals were videotaped with a digital video camera for approximately 2 minutes before the second-stage surgery and then at 1 and 12 weeks after the second-stage procedure (at weeks 13 and 24, respectively). Vibrissal movements were elicited by moving a novel object (eg, a cotton swab or a pencil) toward the rat's head and allowing it to scan or touch the item with a forward sweeping motion of its vibrissae. An 8-second segment of each video recording was identified that best represented vibrissal movement capabilities, and each segment was saved as an uncompressed digital video computer file (720 × 480 pixels, 29.97 frames/s) using Adobe Premiere software (San Jose, Calif).

Four raters who were blind to animal identity and experimental condition watched the video files using a custom program written in Matlab (The MathWorks Inc, Natick, Mass). Video files were presented in random order through a graphic user interface, and raters indicated the degree of symmetry in vibrissal movement using a continuous sliding scale (Figure 2). Video files could be played as many times as needed to make a rating. When the rater pressed the "view new video" button, the current sliding scale value was exported into a spreadsheet program (Excel; Microsoft, Redmond, Wash), and the slider was reset to the midpoint in preparation for rating the next video file.

## HISTOMORPHOMETRIC ANALYSIS

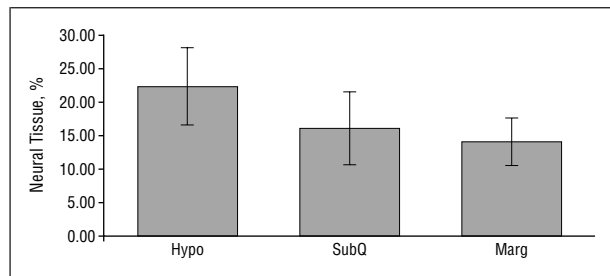
Nerves were fixed in 3% electron microscopy-grade glutaraldehyde and prepared for axon counting according to previously described protocols.<sup>10</sup> Briefly, the fixed implants were serially dehydrated, plastic embedded, cut to 1- $\mu$ m-thick sections, and stained with toluidine blue for axon counting. Measurements were obtained in blinded fashion for fiber number, density, and average diameter, as well as percentage of neural tissue in the total cross-section. Calculations from 10 high-power fields were averaged to obtain mean values for each nerve specimen.

## STATISTICAL ANALYSIS

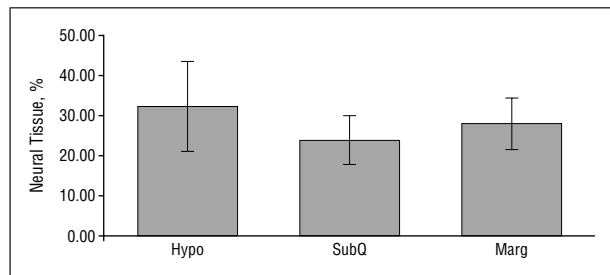
For both functional and histomorphometric findings, comparisons among experimental and control group averages were made using 2-tailed *t* tests ( $\alpha$  level of .05 for all tests), comparing the group baited with the hypoglossal nerve against each of the other 2 groups.

## RESULTS

There were 2 postoperative deaths, both related to anesthesia. There were also 2 instances of mild hindfoot autotomy leading to superficial left lower extremity infections, neither of which resulted in clinically evident systemic illness.



**Figure 3.** Percentage of neural tissue present at week 12 in a cross-sectional area of the distal tip of the sciatic nerve graft in the hypoglossally baited (Hypo), subcutaneously placed (SubQ), and marginally baited (Marg) groups. Error bars indicate 1 SD.



**Figure 4.** Percentage of neural tissue present after 24 weeks in a cross-sectional area of the distal tip of the sciatic graft in the hypoglossally baited (Hypo), subcutaneously placed (SubQ), and marginally baited (Marg) groups. Error bars indicate 1 SD.

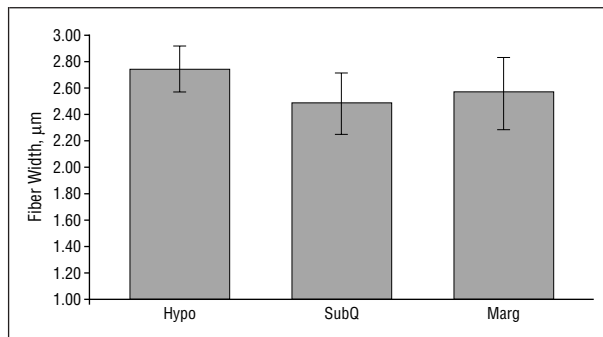
#### HISTOMORPHOMETRIC ANALYSIS

After 12 weeks, analysis of neural ingrowth into the cross-face nerve grafts was performed. In the group in which the graft was baited using the hypoglossal nerve, a mean  $\pm$  SD of 22.31%  $\pm$  5.72% of the cross-sectional area 2 mm from the distal stump of the graft was made up of neural tissue compared with 14.06%  $\pm$  3.49% and 16.10%  $\pm$  5.42% in the marginal and subcutaneously placed grafts, respectively (**Figure 3**). The difference was statistically significant ( $P = .05$ ) between the hypoglossal and marginally baited groups, and trended toward significance between the hypoglossal and subcutaneously baited groups ( $P = .09$ ). The control animals, with no proximal neural input, did not demonstrate neural elements within the grafts.

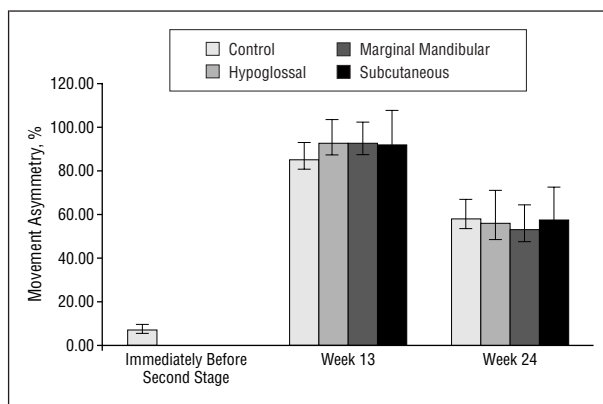
At 24 weeks (12 weeks after second-stage hookup of the distal graft to the left buccal branch of the facial nerve) examination of the neural regenerate through the cross-face nerve grafts of experimental animals revealed that the grafts that had been baited with the hypoglossal nerve continued to have the highest percentage of neural tissue in cross-section, although the difference was no longer statistically significant compared with the subcutaneously and marginally baited animals (**Figure 4**). Determinations of fiber maturity using fiber width measurements demonstrated that the hypoglossally baited group had significantly larger fiber width than the other 2 experimental groups ( $P = .05$ ) (**Figure 5**).

#### BEHAVIORAL ANALYSIS

Before the second procedure, all groups of animals demonstrated bilateral vibrissal movement after 12 weeks, as



**Figure 5.** Average fiber diameter after 24 weeks in the distal buccal branch of the facial nerve in the hypoglossally baited (Hypo), subcutaneously placed (SubQ), and marginally baited (Marg) groups. Error bars indicate 1 SD.



**Figure 6.** Vibrissal function over the second half of the experimental period. On the y-axis, 0 indicates symmetric normal vibrissal function; 100, maximum asymmetry (complete denervation). Error bars indicate 1 SD.

determined by fiber-sweeping symmetry assessment. All animals had at least 1 intact facial nerve branch innervating the vibrissal pad on each side of the face, which confirmed that innervation through either the marginal or the buccal branch of the nerve led to grossly unimpaired vibrissal function.

Seven days after second-stage surgery (which involved transection of the left buccal and left marginal mandibular branches and coaptation of the cross-face nerve graft into the distal buccal branch) all groups demonstrated significant unilateral vibrissal dysfunction (**Figure 6**). At 24 weeks, 12 weeks after the second-stage surgery, vibrissal function had improved significantly ( $P < .05$ ) to roughly 50% of normal in all groups, and there was no significant difference between groups. This included analysis of the control group, in which no deliberate neural input was delivered to the distal stump of either the buccal or the marginal branch of the facial nerve.

#### COMMENT

Current clinical approaches to cross-face nerve grafting involve a 2-stage procedure. In the first stage, a cross-face nerve graft is coapted to several branches of the healthy donor facial nerve and tunneled subcutaneously to the paralyzed side. After a waiting period for neu-

ral ingrowth, the second-stage procedure is performed to coapt the graft to a segment of transferred muscle in the paralyzed side. Functional results following such procedures are highly variable and often disappointing. One of the reasons for poor function after this procedure is suboptimal traversing of axons through the nerve graft. Improvements in functional outcome may result from interventions that promote more robust growth of neural fibers across the graft.

Axonal extension is significantly influenced by the local environment. Both the mechanical and biochemical milieu play a critical role in overall peripheral nerve regeneration.<sup>11-13</sup> Classic experiments, in which the regenerating nerve is inserted into the proximal limb of a Y-shaped conduit and differential growth occurs toward one or the other of the distal 2 limbs based on 2 different distal targets, have established several principles. Experiments involving a straight conduit with different distal targets corroborate these findings. The regenerating stump will preferentially choose a neural target over nonneural tissues,<sup>7-9</sup> and the attractiveness of the neural target is volume and surface-area dependent.<sup>14</sup> Moreover, when the distal targets contain metabolically active nerve vs nerve that has been biochemically treated to halt metabolic activity, the metabolically active target is favored by the regenerating nerve.<sup>8</sup>

Investigators have attempted to exploit the distal target sensitivity of the regenerating nerve to achieve more robust regeneration. Mackinnon et al<sup>15</sup> showed that nerve regeneration across a long graft in a monkey facial nerve model was more robust when the distal end of the nerve graft was sutured to the recipient facial nerve stump rather than being left in the subcutaneous space. Whether long-term reinnervation of the face would differ using an immediate vs a delayed hookup of the distal end of the cross-face nerve graft was not addressed. In a similar study of a long nerve graft involving an end-to-side coaptation in the rat hind limb, Goheen-Robillard et al<sup>16</sup> demonstrated that significantly more axons traversed the graft when the distal end was secured to a neural target than when it was left in the subcutaneous space. Studies in other species reveal a relationship between the number of myelinated axons present in the distal stump of a long autograft with no distal muscle hookup and the environment of the nerve graft (ipsilateral vs contralateral extremity).<sup>17,18</sup> The species-specific differences in number of axons traversing a long autograft, the effect of target muscle hookup, and the inferiority of axonal traversing in humans have been described.<sup>19</sup>

Distal targets exert their neurotrophic effects via the elaboration of soluble neurotrophic factors. Sources for these factors include the Schwann cells in the distal stump, the breakdown products of nerve after Wallerian regeneration, and the distal denervated musculature.<sup>20-22</sup> The present study compared the combined neurotrophic effect of (1) distal hookup to a nerve/muscle complex rich in motor fibers (hypoglossal stump), (2) distal hookup to a nerve/muscle complex with significantly fewer motor fibers (marginal branch of facial nerve stump), and (3) no distal hookup in the rat cross-face nerve graft model. The results indicate that while baiting the cross-face nerve graft with temporary hypoglossal hookup did result in higher axon counts

and more mature fibers through the graft compared with the other groups, this effect did not ultimately provide superior anatomic or functional recovery following take-down of the neurotomy and coaptation to the ultimate distal target, the buccal branch of the facial nerve.

Immediately prior to the second-stage surgery, all animals had bilateral vibrissal movement because all animals had at least 1 facial nerve branch intact on both sides of the face. There was no obvious right/left asymmetry in vibrissal movement in animals that received partial vibrissal denervation 12 weeks earlier, probably because of overlap in rat buccal and marginal facial nerve innervation patterns and peripheral sprouting and reinnervation of denervated muscle fibers that occurred during the first-stage recovery period.

Raters consistently observed asymmetry in the right vs left vibrissal movements recorded 1 week after the second-stage surgery (week 13) and a significant return toward symmetry of movement by the end of 24 weeks for all groups. At the 13- and 24-week periods, there were no significant differences among experimental and control group averages for movement ratings. This is to be expected at the 13-week time point because the cross-face nerve grafts were freshly connected to the distal buccal VII branch (experimental) or were cut and left in the subcutaneous space (control). However, since the control group showed the same degree of functional recovery as the experimental groups at 12 weeks and yet had almost no neural elements present in distal cross-face grafts at the time of the animal's death, this suggests that significant reinnervation from cut facial nerve ends occurred in control animals and therefore possibly in experimental animals as well. This was further supported by the observation in 1 control animal of electrical stimulation of the proximal facial nerve trunk leading to vibrissal movement at the time of death. It is possible that this unexpected sprouting from the transected facial nerve stumps dominated the recovery and thus concealed the potential functional differences between the higher-quality neural regeneration in the hypoglossally baited group and the other 2 experimental groups.

Our histomorphometric and functional data are consistent with prior studies of regeneration through long segments of autograft nerve.<sup>15,16</sup> Distal coaptation to a target nerve/muscle complex enhances the quality of neural regeneration through the graft. Whether there is a mechanism for using these baiting techniques to substantially improve histomorphometric and functional parameters must be further investigated. A critical element for future studies will be to eliminate potential native facial nerve sprouting by performing more complete neurectomies on the side of interest. This could be accompanied by burying the facial nerve stump into the masseter muscle to further prevent the likelihood of fibers "finding" their distal targets.

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## REFERENCES

1. Harii K, Ohmori K, Torii S. Free gracilis muscle transplantation with microvascular anastomoses for the treatment of facial paralysis: a preliminary report. *Plast Reconstr Surg*. 1976;57:133-143.
2. Harrison D. Current trends in the treatment of established unilateral facial palsy. *Ann R Coll Surg Engl*. 1990;72:94-98.
3. O'Brien B, Pederson W, Khazanchi R, et al. Results of management of facial palsy with microvascular free muscle transfer. *Plast Reconstr Surg*. 1990;86:12-22.
4. Manktelow R. Free muscle transplantation for facial paralysis. *Clin Plast Surg*. 1984;11:215-220.
5. Zuker R, Goldberg C, Manktelow R. Facial animation in children with mobius syndrome after segmental gracilis muscle transplant. *Plast Reconstr Surg*. 2000;106:1-8.
6. Hadlock T, Varvares M. Microvascular and microneural surgery: new horizons in facial plastic surgery. *Facial Plast Surg Clin North Am*. 2002;10:167-174.
7. Lundborg G, Dahlin L, Danielsen N, et al. Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol*. 1982;76:361-375.
8. Politis M, Ederle K, Spencer P. Tropism in nerve regeneration in vivo: attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. *Brain Res*. 1982;253:1-12.
9. Tos P, Battiston B, Geuna S, et al. Tissue specificity in rat peripheral nerve regeneration through combined skeletal muscle and vein conduit grafts. *Microsurgery*. 2000;20:65-71.
10. Hadlock T, Sundback C, Hunter D, et al. A new artificial nerve graft containing rolled Schwann cell monolayers. *Microsurgery*. 2001;21:96-101.
11. Seckel B. Enhancement of peripheral nerve regeneration. *Muscle Nerve*. 1990;13:785-800.
12. Pollock M. Nerve regeneration. *Curr Opin Neurol*. 1995;8:354-358.
13. Lundborg G. A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J Hand Surg (Am)*. 2000;25:391-414.
14. Takahashi Y, Maki Y, Yoshizu T, Tajima T. Both stump area and volume of distal sensory nerve segments influence the regeneration of sensory axons in rats. *Scand J Plast Reconstr Surg Hand Surg*. 1999;33:177-180.
15. Mackinnon S, Dellon A, Hunter D. Histological assessment of the effects of the distal nerve in determining regeneration across a nerve graft. *Microsurgery*. 1988;9:46-51.
16. Goheen-Robillard B, Myckatyn T, Mackinnon S, Hunter D. End-to-side neurotomy and lateral axonal sprouting in a long rat graft model. *Laryngoscope*. 2002;112:899-905.
17. Frey M, Koller R, Gruber I, et al. Time course of histomorphometric alterations in nerve grafts without connection to a muscle target organ: an experimental study in sheep. *J Reconstr Microsurg*. 1992;8:345-357.
18. Koller R, Frey M, Meier U, et al. Fiber regeneration in nerve grafts without connection to a target muscle: an experimental study in rabbits. *Microsurgery*. 1993;14:516-526.
19. Frey M, Koller R, Liegl C, Happak W, Gruber H. Role of a muscle target organ on the regeneration of motor nerve fibers in long nerve grafts: a synopsis of experimental and clinical data. *Microsurgery*. 1996;17:80-88.
20. Bunge R. Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. *Curr Opin Neurobiol*. 1993;3:805-809.
21. Frostick S, Yin Q, Kemp G. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. *Microsurgery*. 1998;18:397-405.
22. Bryan D, Wang K, Summerhayes C. Migration of Schwann cells in peripheral nerve regeneration. *J Reconstr Microsurg*. 1999;15:591-596.