Functional Recovery After Facial and Sciatic Nerve Crush Injury in the Rat

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Objectives: To systematically record rat facial nerve recovery following crush injury to the main trunk with respect to ocular and vibrissial function and to compare the rates of facial and sciatic nerve recovery from crush injury in the same animals. This serves as a means of validating the functional parameters of facial nerve recovery against the well-known measure of hind limb function, the Sciatic Function Index.

Methods: The main trunk of the facial nerve and the proximal segment of the sciatic nerve were exposed in all animals. Both nerves were subjected to standardized crush injury and subsequent daily functional testing. After a plateau of functional recovery was achieved, the animals were killed, and the distances between the sites of injury and the end musculature were measured, which allowed determination and comparison of recovery rates in both systems.

Results: All crush injuries resulted in loss of electrical conductivity, as proven by intraoperative proximal nerve stimulation. Recovery of ocular and vibrissial motor function occurred starting at postoperative day (POD) 9 and continuing through POD 20. Hind limb function returned later (POD 14-34); however, when corrected for distance, the sciatic recovery rate (2.26 mm/d) appeared to match that of the facial nerve (1.5-2.4 mm/d).

Conclusions: Recovery after facial nerve crush injury follows a predictable time course, and the rate of recovery is consistent with that of sciatic nerve injury. Return of the blink reflex, loss of vibrissial fibrillations, and return of vibrissial sweeping function appear to be internally consistent functional measures of facial recovery. These quantitative measures will be useful for future facial nerve manipulation studies.

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AS THE NERVE-SPECIFIC REGENERATIVE BIOLOGY OF CRANIAL AND OTHER PERIPHERAL NERVES HAS BECOME APPRECIATED, INTEREST HAS Risen in developing models of nerve regeneration that are pertinent to particular clinical questions. Investigations into facial nerve recovery have used rodent and other small animal models as well as large animal models, but there has not been a universal assessment of functional recovery that has been tested and validated against well-developed measures of functional recovery in rat nerve. The development of a standard model of rodent facial nerve injury and recovery would be of significant value to the otolaryngologist—head and neck surgeon or plastic surgeon interested in facial nerve regeneration.

The objective of this study was to determine the rate of functional recovery of both the orbicularis oculi muscle and the vibrissial motor pad of the rat facial nerve following crush injury. In addition, by creating a sciatic nerve injury in the same animals and measuring the rate of hind limb recovery, we were able to compare rates of recovery in a cranial nerve of great clinical interest with those of a frequently studied spinal motor nerve. Correlation between these rates of recovery within the same animal established the validity of measuring orbicularis oculi and vibrissial motor pad function to predict the return of facial nerve fibers into motor end plates after crush injury. The establishment of standard curves of recovery rate following crush injury to the facial nerve creates a platform on which to develop recovery curves following transection, the bridging of gap defects via entubulation, and various other biological and physical manipulations.

METHODS

SURGICAL PROCEDURE

Ten male Sprague-Dawley rats weighing 350 to 490 g were allowed to acclimate to the walking track test setting for several days preoperatively. On the day of surgery, animals were anesthetized with a subcutaneous injection of...
75 mg/kg of ketamine hydrochloride (Fort Dodge Animal Health, Fort Dodge, Iowa) and 0.5 mg/kg of medetomidine hydrochloride (Orion Corporation, Espoo, Finland). The left hind limb and left infraauricular areas were shaved and steriley prepared. The sciatic nerve was exposed at the sciatic notch and crushed for 30 seconds using a jeweler's microforceps. The crush injury was then repeated for an additional 30 seconds in the same location. The proximal nerve was stimulated with a Montgomery Nerve stimulator (Boston Medical Products, Westford, Mass) at a setting of 2 mV to verify loss of electroconductivity. If there was persistent motor function, the nerve was crushed a third time (n = 2; 20%), and the loss of electrical conductivity was verified by repeated proximal stimulation. The hind limb wound was closed.

The facial nerve was approached through an infraauricular incision. The main trunk of the facial nerve was identified as it emerged anterior to the posterior belly of the digastric muscle (Figure 1) and electrically stimulated to verify entire hemifacial movement. The nerve was then double-crushed as described above, and loss of electrical conductivity was verified. The wound was closed in a single layer, and the anesthetic was reversed with a subcutaneous injection of 0.05 mg/kg of atipamezole hydrochloride. The Massachusetts Eye and Ear Infirmary animal care guidelines were strictly followed.

RECOVERY ANALYSIS

Starting on the fifth postoperative day, animals underwent daily functional testing. Walking track analysis was performed by dipping the feet into a methylene blue solution (3% w/w in water) and permitting the animal to ambulate down a standard walking track into which a strip of white paper had been placed. Measurements were made from the prints according to the method of Bain et al, and the Sciatic Function Index (SFI) was computed based on the described formula. Animals were then sedated with an intramuscular injection of 0.5 mg/kg of medetomidine hydrochloride and placed on a black surface so that the face was centered in a circle defined by a 12-inch ring fluorescent light bulb directly under a digital video camera mounted on a tripod.

Denervated sling muscle fibers (controlling vibrissial sweeping) caused observable movement of the vibrissae when the muscle fibers fibrillated, and this movement was video recorded. A drop of water was then placed into each control eye (right eye) to determine the presence of the blink reflex. Once the presence of the blink was verified, establishing the lack of oversedation, the experimental eye (left eye) received a drop, and the presence or absence of blinking effort was recorded. A reversing injection of 0.05 mg/kg of atipamezole hydrochloride was then administered, and the volitional vibrissal movements were assessed immediately on emergence from sedation for presence and symmetry.

SCORING OF FACIAL FUNCTION

Rating of specific movements proceeded as follows: The presence of fibrillation-related movement of all ipsilateral vibrissae was given a score of 0. Complete absence of fibrillations was given a score of 1, indicating full reinnervation. When there appeared to be only a percentage of vibrissae fibrillating, a decimal rank was assigned corresponding to the number of vibrissae with loss of fibrillations (recovered vibrissae). For example, when approximately 90% of the vibrissae appeared to have lost fibrillations, a score of 0.9 was assigned. Ocular function was scored based on the degree of eye closure, or palpebral fissure narrowing, with drop stimulation: 0 indicated no eye closure; 1, complete eye closure; and 0.5, 50% narrowing of the palpebral fissure on stimulation. For recovery of vibrissal sweeping, the same scale was applied as for fibrillation-related movement: 0 indicated complete asymmetry with no movement of vibrissae on the affected side; 1, symmetric vibrissal sweeping; and decimal scores represented the degree of vibrissal sweeping symmetry.

Twenty-one days postoperatively, when facial function had returned, animals underwent only walking track analysis. This continued every several days until postoperative day 36, when a clear plateau of recovery had been established.

Video recordings of vibrissal and ocular movement were analyzed first at the time of acquisition and later by 2 independent blinded observers. Average functional scores were established from the 3 readings, and curves of percentage recovery vs postoperative day were plotted.

Animals were allowed to survive 40 days, after which they were killed by inhalational isofluorane overdose. The sciatic and facial nerves were reexposed, and the length of nerve between the crush injury and the entrance of the posterior tibial nerve into the foot musculature was measured. The distances from the facial nerve crush site to the medial canthus and from the crush site to the center of the vibrissal pad were recorded.

Based on these distance measures, and taking the plateau values for both facial and sciatic function as the complete recovery mark, rates of recovery were calculated and reported in millimeters per day. These values were compared using a 2-tailed t test for between-group comparisons (α = .05).

RESULTS

There were no intraoperative complications. In 2 animals, 3 sciatic nerve crushes were required to achieve complete loss of electroconductivity. One animal experienced mild postoperative autotomy and did not have meaningful footprint data from which to compute SFI despite the use of Bitter Apple taste deterrent (Granicks, Greenwich, Conn).

FACIAL NERVE RECOVERY

Loss of vibrissal fibrillations indicating reinnervation was complete by postoperative day (POD) 19.2 on average.
Vibrissial sweeping movements returned along a slightly earlier time course, with complete return by POD 16 (Figure 3). Eyelid blink reflex was the first of the facial parameters to show evidence of recovery (POD 9), but it did not completely recover until POD 18 on average (Figure 4). By POD 20, stable facial function was fully recovered.

The average distances from the main trunk of the facial nerve to the medial canthus and to the vibrissial pad were 31.5 mm and 42.8 mm, respectively. Assuming that a plateau of recovery (≥92%) indicated arrival of a critical number of fibers in the muscle of interest, calculations of the rate of recovery were performed for each animal and averaged (Figure 5). The upper division of the facial nerve regenerated slower than the buccal branch (mean ± SD rates of regeneration, 1.75 ± 0.16 mm/d and 2.24 ± 0.4 mm/d, respectively; P<.001).

**HIND LIMB RECOVERY**

Walking track analysis of prints from the animals revealed an average early postoperative deficit of −99.6%, indicating successful complete denervation. Recovery reached a plateau by POD 34 at a level of −20%, in solid agreement with the recovery level found after crush injury in other studies.1-3

The mean ± SD length of the sciatic nerve from crush site to nerve entry into foot musculature was 74.4 ± 8.0 mm. The rate of nerve regeneration was 2.20 ± 0.3 mm/d, which was not significantly different than the value found for buccal branch regeneration (P = .73)(Figure 5).

**COMMENT**

Most investigations into the effects of facial nerve damage have focused on changes that occur centrally (facial motor nucleus or cortex). Many investigators have studied the somatotopic organization of the facial nucleus and ways in which these patterns change after crush injury or transection of the facial nerve.4-9 For example, retrograde tracer studies have quantified the relative number of misguided axons8,9 and the degree of hyperinnervation following facial nerve main trunk injury.10,11 Investigators have also studied changes in facial muscle fibers following denervation as well as changes in cortical representation of the face after facial nerve injury.

There have been a modest number of reports of the recovery of facial function in the rodent following facial nerve manipulation. Most of these studies use a 3- or 4-point grading scale to rate vibrissal movement symmetry following mouse facial nerve injury.12-17 To date, only a single study has described the different zones of facial function independently.18 Another group has developed a quantitative functional recovery scale based on precise videographic recordings of vibrissal movement in the rat.8

Our present investigation demonstrates that the function of different groups of facial muscles can be recorded videographically and ranked. Video documentation of facial movements permits blinded analysis of facial function according to specific continuous grading scales. We have shown herein that it is possible to follow ocular function independently of midfacial function by test-
ing the blink reflex independently of vibrissal movement. It is also possible to crop the video images to include only ocular or mid facial regions so that the 2 zones of the face, representing 2 separate branches of the facial nerve, can be analyzed separately.

We found that the return of symmetric sweeping motion in the vibrissal whisker pad returns earlier than the complete disappearance of fibrillations. Previous investigators using retrograde neural pathway tracing in conjunction with functional assessment have found that sweeping vibrissae can appear symmetrical (eg, normal) even when approximately 44% of motor axons are disrupted after crush injury. Therefore, substantial reinnervation may persist beyond the point in recovery where sweeping movement appears normal, generating continued fibrillation-related vibrissal movement until reinnervation is more complete. We conclude from this that the loss of vibrissal fibrillations is a more accurate measure of reinnervation of the vibrissal musculature than vibrissal sweep, and we will use this measure in the future as a marker of significant reinnervation. Others have noted the utility of monitoring fibrillation-related vibrissal movement for tracking rodent facial nerve regeneration (James Heaton, PhD, and James Kobler, PhD, unpublished data, 2004).

We found that partial ocular function was the first to appear in the recovery period. This was anticipated because the upper division of the facial nerve follows the shortest course of any of the anterior nerve branches before entering the orbicularis oculi in the region of the lateral canthus. We also found that the time course until complete recovery of the blink was delayed with respect to the return of symmetrical vibrissal sweeping and more closely approximated the disappearance of fibrillation-related movement. This may indicate that proportionally more muscle fibers are required for ocular function than for vibrissal sweeping function, although this issue was not answered by the current study.

Sciatic function returned according to a predictable time course commensurate with that found by other sciatic crush investigations. The conclusions regarding rate of nerve regeneration were calculated from distance measures of the sciatic nerve crush injury site to muscle entry point coupled with the SFI data. The calculations were based on the assumption that the arrival of fibers into foot musculature corresponds with changes in the SFI. There was excellent agreement in mean ± SD recovery rate between the sciatic nerve (2.20 ± 0.3 mm/d) and the buccal branch of the facial nerve (2.24 ± 0.4 mm/d) when loss of vibrissal fibrillations was used as the marker of recovery.

To date, there is no well-accepted method for determining functional recovery after rat facial nerve manipulation. The present study used a semiquantitative analysis of the function of different facial muscles after facial nerve crush injury and compared the rate of regeneration with that of sciatic function after a similar crush injury. The finding that buccal branch recovery follows the same time course as sciatic nerve recovery supports the concept that peripheral nerves, whether cranial or spinal, regenerate at roughly the same rate following crush injury. Therefore, techniques of proven benefit in the study of the sciatic nerve might prove useful as well in the study of the facial nerve.

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