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 vibrissal 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 vibrissal 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 vibrissal fibrillations, and return of vibrissal 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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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...
muscle fibers fibrillated, and this movement was video re-
ing) caused observable movement of the vibrissae when the

on a tripod.

the face was centered in a circle defined by a 12-inch ring fluo-

detomidine hydrochloride and placed on a black surface so that

sedated with an intramuscular injection of 0.5 mg/kg of me-

computed based on the described formula. Animals were then

animal care guidelines were strictly followed.

zole hydrochloride. The Massachusetts Eye and Ear Infirmary

reversed with a subcutaneous injection of 0.05 mg/kg of atipame-

scribed above, and loss of electrical conductivity was verified.

facial movement. The nerve was then double-crushed as de-

incision. The main trunk of the facial nerve was identified as it

approached through an infraauricular

approach to the main trunk of the facial nerve and location of the

Figure 1. Approach to the main trunk of the facial nerve and location of the crush site. The black line indicates the skin incision; yellow lines, facial nerve anatomy; and red lines, the contour of the muscle belly.

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 hemi-

facial movement. The nerve was then double-crushed as de-

scribed 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 atipame-

zole hydrochloride. The Massachusetts Eye and Ear Inflammatory

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 dip-

ping the feet into a methylene blue solution (3% w/w in wa-

ter) 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,1 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 me-

detomidine hydrochloride and placed on a black surface so that

the face was centered in a circle defined by a 12-inch ring fluo-

scent light bulb directly under a digital video camera mounted

on a tripod.

Denervated sling muscle fibers (controlling vibrissial sweep-

ing) caused observable movement of the vibrissae when the

muscle fibers fibrillated, and this movement was video re-
corded. 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 hydrochlor-

dide was then administered, and the volitional vibrissial move-

ments were assessed immediately on emergence from seda-

tion for presence and symmetry.

SCORING OF FACIAL FUNCTION

Rating of specific movements proceeded as follows: The pres-

cence of fibrillation-related movement of all ipsilateral vibris-

sae 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 deci-

mal rank was assigned corresponding to the number of vibris-

sae with loss of fibrillations (recovered vibrissa). For ex-

ample, when approximately 90% of the vibrissa appeared to

have lost fibrillations, a score of 0.9 was assigned. Ocular func-

tion was scored based on the degree of eye closure, or palpe-

bral 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 vibris-

sial 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 vi-

brissial 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 vibrissial and ocular movement were

analyzed first at the time of acquisition and later by 2 indepen-

dent blinded observers. Average functional scores were estab-

lished 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 be-

tween 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 re-

covery 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 ani-

mals, 3 sciatic nerve crushes were required to achieve com-

plete 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 (Gran-

nicks, Greenwich, Conn).

FACIAL NERVE RECOVERY

Loss of vibrissial 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. For example, retrograde tracer studies have quantified the relative number of misguided axons and the degree of hyperinnervation following facial nerve main trunk injury. 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 vibrissial movement symmetry following mouse facial nerve injury. To date, only a single study has described the different zones of facial function independently. Another group has developed a quantitative functional recovery scale based on precise videographic recordings of vibrissial movement in the rat.

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-

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**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. For example, retrograde tracer studies have quantified the relative number of misguided axons and the degree of hyperinnervation following facial nerve main trunk injury. 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.
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.\textsuperscript{14} Therefore, substantial denervation may persist beyond the point in recovery where sweeping motion 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.\textsuperscript{1-3} 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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REFERENCES


