Facial palsy resulting from facial nerve (FN) injury affects up to 10% of patients undergoing removal of cerebellopontine angle tumors. The loss of facial symmetry and expression following facial palsy has a great impact on psychosocial conditions of the patients. Hypoglossal nerve–facial nerve neurorrhaphy is a conventional method for treating complete facial palsy (CFP) when the proximal FN stump is not available. Since complete transection of the hypoglossal nerve (HN) may result in paralysis and atrophy of the ipsilateral hemitongue, HN-FN “side”-to-end neurorrhaphy by using only one-half of the HN is preferable to their end-to-end neurorrhaphy. Incomplete facial palsy (IFP), which has a relatively high prevalence among patients who undergo cerebellopontine angle surgery, results from FN injury with remnant axons or insufficient spontaneous axonal regeneration. For the patients with persistent and important facial deficits, hypoglossal-facial nerve “side”-to-side neurorrhaphy for persistent incomplete facial palsy is used as an alternative method.

**Laboratory investigation**

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**Object.** Hypoglossal-facial nerve neurorrhaphy is a widely used method for treating complete facial palsy. However, the classic surgical procedure using a “side”-to-end neurorrhaphy is not suitable for incomplete facial palsy (IFP), because sectioning of the facial nerve for neurorrhaphy compromises remnant axons and potential spontaneous reinnervation. For the treatment of persistent IFP, the authors investigated in rats a modified method using hypoglossal-facial nerve “side”-to-side neurorrhaphy.

**Methods.** An IFP model was created by crushing the facial nerve and then ligating the injury site to limit axonal regeneration. After 9 weeks, rats with IFP were submitted to hypoglossal-facial nerve “side”-to-side neurorrhaphy: The gap between the 2 nerves was bridged with a predegenerated peroneal nerve graft, which was sutured to only one-half of the hypoglossal nerve and to the remnant facial nerve through a small window created by removing the epineurium, thus preserving regenerating facial axons.

**Results.** Four months after repair surgery, double innervation of the target whisker pad by hypoglossal and facial motor neurons was supported by the recording of muscle action potentials and their retrograde labeling. Regenerated hypoglossal and facial motor neurons effectively participated in the reinnervation of the whisker pad, significantly improving facial symmetry without evident synkinesis, compared with rats that underwent IFP without hypoglossal-facial nerve neurorrhaphy.

**Conclusions.** This study demonstrates that hypoglossal-facial nerve “side”-to-side neurorrhaphy with a predegenerated nerve graft can lead to rapid functional benefits for persistent IFP without compromising the remnants of facial axons, thus providing a proof-of-feasibility for further studies in humans.

**Key Words** • facial symmetry • facial reanimation • nerve graft • nerve regeneration • peripheral nerve injury • surgical technique
surgical repair of the injured FN is desirable. However, the classic HN-FN “side”-to-end neurorrhaphy is not suitable for these patients because complete sectioning of the FN for neurorrhaphy compromises its remnant axons and/or spontaneous reinnervation potential. We thus assessed a modified method for the treatment of persistent IFP by using a HN-FN “side”-to-side neurorrhaphy through a pre-degenerated nerve graft (PNG). The “side”-to-side neurorrhaphy was achieved through a window at the injured FN where only the epineurium was removed. This intervention does not interrupt the main structure of the FN at the neurorrhaphy site, thereby preserving the remnants of facial axons and their potential for spontaneous reinnervation.

A rat model with persistent IFP was created by performing a crush injury of the FN and then ligating the injury site to limit axonal regeneration. Nine weeks after injury, functional evaluation was performed to select rats that developed IFP. In those rats, the HN-FN “side”-to-side neurorrhaphy was performed. The animals were then followed up for 4 months and functional, electrophysiological, and histological examinations were performed.

Methods

Experiments were approved by the local animal care committee and were performed by authorized investigators in accordance with French law.

Facial Nerve Injury and Reconstruction

Thirty-four male Fisher-344 rats (Charles River, France) 6 weeks of age were used as experimental (n = 12), control (n = 12), intact (n = 4), and donor (n = 6) animals in this study. Rats underwent general anesthesia through an intraperitoneal injection of pentobarbital (72 mg/kg) for surgical intervention. For FN injury, the right FN was exposed under a surgical microscope (Leica M650). After the recording of muscle action potentials (MAPs) in the right whisker pad in response to FN electrostimulation using a Myto electromyography unit (EB-Neuro), we used microforceps to crush-injure the FN; this was done by sectioning all of the axons except the nerve’s perineurium at the site close to the nerve’s emergence from the stylomastoid foramen but distal to its posterior auricular branch, resulting in the total degeneration of the distal FN (Fig. 1A and B). The injury site was then ligated with 4-0 nylon sutures. In preliminary studies, this intervention resulted in spontaneous but limited axonal regeneration. The surgical wound was closed in layers with 4-0 nylon sutures, and amitriptyline (15 mg/kg/day) was added to the rats’ drinking water for 2 weeks to reduce their neuropathic pain. Nine weeks later, MAPs were recorded again. MAP surface values that were reduced to approximately 10%–20% of their initial preinjury values qualified as persistent IFP. Rats with IFP underwent no repair surgery as controls (IFP rats, n = 6), representing spontaneous incomplete regeneration, or were subjected to HN-FN “side”-to-side neurorrhaphy using a 10-mm PNG (IFP-R rats, n = 12). Their right HN was exposed beneath the digastric muscle, and about 50% of its axons were cross-sectioned. One end of a PNG was anastomosed end-to-“side” to the HN at the partial injury site.
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using 10-0 nylon microsutures. The other end of the PNG was anastomosed end-to-side to the distal stump of the injured FN through a window where only the epineurium was removed (Fig. 1C).

Depending on the suitability of their diameter for the neurorrhaphy with HNs and FNs,36 PNGs were prepared from peroneal nerves of donor Fisher-344 rats (n = 6). Briefly, peroneal nerves were exposed and sectioned as close as possible to their origin from the sciatic nerve, leading to distal axonal degeneration and Schwann cell proliferation. One week later, the distal part of the lesioned peroneal nerve (10 mm long) was removed and used as a PNG, providing an environment supporting axonal regeneration.6,19 Because the Fisher-344 rats are syngeneic, the PNGs were considered as autografts, and no signs of immune responses or rejection were found in recipient rats.

For comparison with CFP, an additional control group of rats (n = 6) underwent sectioning of the FN followed by a double ligature, preventing spontaneous regeneration. CFP rats received no surgical repair.36

The progress of all animals was followed for a period of 4 months, from the time of 9 weeks after FN injury. At the end of the follow-up period, facial symmetry analysis and electrophysiological monitoring were performed before and after sectioning the partially regenerated right FN of IFP rats and either the right FN proximal to the neurorrhaphy site or the PNG of IFP-R rats. The delay between the 2 examinations was 1 week. Three days before the electrophysiological examination prior to sectioning of the FN or PNG, neuronal tracers were injected into the rats for subsequent retrograde labeling studies. The rats were euthanized with an overdose of pentobarbital (120 mg/kg) so that histological examination could be conducted after the last electrophysiological measure. IFP-R rats that underwent either FN or PNG cutting are presented as independent groups throughout the experiment: “IFP-R cut FN” and “IFP-R cut PNG” (n = 6 each). We also used 4 intact rats for histological examination to obtain normal data on retrograde labeling as well as optic and electron microscope studies.

Behavioral Testing

Each rat was photographed before and after surgery, and the Angle α between a line extending from the fold on the bridge of the nose and a line linking the outer corners of each of the eyes was measured (Fig. 2A).36 Measuring Angle α allows determination of changes in facial resting-tone symmetry. Before and after surgery video recording was performed to observe behaviors and synchrony during eating and drinking. Body weight was recorded to assess the HN function related to feeding after surgery. All photographing, video recording, and analysis were performed in a blind manner. To assess which nerve pathways contributed to the recovery of facial symmetry at the end of the follow-up period, the PNG or the FN proximal to the bridged PNG was sectioned, and Angle α was measured again. The measurements of Angle α in the posttraumatic period were determined 1 week after injuring the FN at the initial surgery or sectioning the PNG or FN at the end of follow-up period to allow the rats recovery from surgery and anesthesia.

Electrophysiological Examination

We recorded MAPs using electromyography simultaneously at the right whisker pad and the hemitongue muscles during direct electrostimulation of either the FN or HN trunk or the PNG. Stimulation (0.1 ms, 0.3 mA) was delivered through 2 0.5-mm-diameter electrodes directly placed onto the injured FN, the PNG, or the HN proximal to the neurorrhaphy site. The MAPs were recorded by 2 other 0.5-mm-diameter electrodes inserted into the right whisker pad and the hemitongue. The amplitudes between the largest positive and negative peaks and the surface beneath the slope were measured. The latency was not measured because of its lack of reliability for such a short conduction distance. After 4 months of recovery following nerve repair, the PNG or the FN proximal to the bridged PNG was sectioned, and the electrostimulation was then immediately performed again to check which nerve pathway was responsible for active MAPs.

Retrograde Labeling Study

At the end of the 4-month follow-up period, retrograde labeling with fluorescent tracers was performed in rats to detect regenerated motor neurons in the related hypoglossal and facial nuclei. Rats were reanesthetized and 20 μl of 1% cholera toxin subunit B conjugated with Alexa Fluor 555 (CTB–Alexa 555) was injected into the right whisker pad at multiple points, while 10 μl of 2% diamidino yellow (DY) was injected into the right hemitongue. Ten days later, rats were killed by intraperitoneal overdose injection of pentobarbital (120 mg/kg) and were perfused with 300 ml phosphate-buffered saline (0.1 M, pH 7) followed by 300 ml 4% paraformaldehyde. The brainstem was removed and postfixed in the parafomaldehyde fixative solution for 3 hours. The specimens were then immersed in 30% sucrose at 4°C for cryopreservation. Cross-sections (30 μm) were cut with a freezing microtome. Using a Zeiss AxioPlan 2 imaging optic fluorescence microscope (200 M), labeled neurons were identified and counted in all sections covering the facial and hypoglossal nuclei.

Optic and Electron Microscope Analysis

Axonal elongation in the PNG and FN was assessed by optical and electron microscopy. The PNG and FN in each animal were removed at the end of the follow-up period and fixed in 3.6% glutaraldehyde for 3 hours. The specimens were postfixed with osmium tetroxide and then embedded in Epon. Semi-thin (0.35-μm) and ultra-thin (0.07-μm) cross-sections were acquired using an ultra-microtome (Reichert Ultracut S Wild M3z, Leica) and stained with thionin or uranyl acetate and lead citrate, respectively. Semi-thin sections were examined under an optic microscope, and ultra-thin sections were analyzed with a 1200ExII transmission electron microscope (JEOL).

Statistical Analysis

Group differences were analyzed using 2-way or 1-way ANOVA followed by Newman-Keuls post hoc
tests. Data are presented as mean ± SEM. Statistica 64 software, version 10 (StatSoft) was used.

Results

Recovery of Facial Symmetry After HN-FN “Side”-to-Side Neurorrhaphy

Changes in facial symmetry were analyzed by measuring Angle $\alpha$ (Fig. 2A). This angle corresponded to an average of 90° in intact rats, whereas it decreased to about 71° 1 week after FN injury in rats with persistent IFP or CFP (Fig. 2B). It then remained unchanged in CFP rats until the end of the follow-up period; however, at 9 weeks postinjury, a slight recovery was observed in IFP rats (average angle 74°), indicating spontaneous regeneration and partial reinnervation by FN axons. In the absence of repair surgery (IFP rats), Angle $\alpha$ remained at an average of 74°. However, significantly higher values (p < 0.01), ranging from 76° to 80°, were observed 4 months after the HN-FN “side”-to-side neurorrhaphy using a PNG (IFP-R rats) (Fig. 2B).

When the PNG was sectioned in IFP-R rats at the end of the follow-up period (IFP-R cut PNG), the Angle $\alpha$ that was measured after 1 week decreased to the values observed before HN-FN neurorrhaphy was performed. When the FN was cut in IFP-R rats proximal to the neurorrhaphy site (IFP-R cut FN), the average Angle $\alpha$ decreased to 76°. In IFP rats that did not undergo repair surgery, Angle $\alpha$ decreased to about 71° after sectioning of the FN (Fig. 2B).

Nerve Conduction in the Reconstructed Pathways

To assess functional target innervation, MAPs were recorded in the whisker pad. In response to electrostimulation of the FN before injury, MAPs exhibited values of 6.97 ± 1.14 mV for the amplitude and 6.05 ± 0.96 mV msec (millivolts times milliseconds) for the surface (Fig. 3). They disappeared completely after FN injury and were not recordable in CFP rats at any time after injury. However, 9 weeks after injury, MAPs could be again recorded (0.9 ± 0.54 mV for the amplitude and 1.11 ± 0.5 mV msec for the surface) in IFP rats, indicating spontaneous reinnervation. The MAP values increased to 3.29 ± 0.8 mV for the amplitude and 3.45 ± 0.71 mV msec for the surface in IFP-R rats 4 months after the HN-FN neurorrhaphy (p < 0.01), whereas they remained unchanged in IFP rats in the absence of repair surgery (Fig. 3).

Muscle action potentials could also be recorded in the whisker pad of IFP-R rats when the PNG was directly stimulated (amplitude 1.52 ± 0.47 mV, surface 1.63 ± 0.39 mV).
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Importantly, evident muscle contraction was observed in the right whisker pad but not in the ipsilateral hemitongue when the PNG was intensely stimulated, indicating that the hypoglossal axons growing through the PNG selectively innervated facial muscles (Video 2).

Sectioning of the PNG at 4 months after repair surgery (IFP-R cut PNG rats) resulted after 1 week in a significant decrease in whisker pad MAPs in response to FN electrostimulation (amplitude 1.36 ± 0.56 mV, surface 1.38 ± 0.45 mVmsec) (Fig. 3). Muscle action potentials also decreased when the FN was sectioned proximal to the neurorrhaphy site in IFP-R rats (that is, the IFP-R cut FN; amplitude 1.69 ± 0.66 mV, surface 1.88 ± 0.61 mVmsec). In the absence of repair surgery, MAPs disappeared completely in IFP rats at the end of the follow-up period, confirming the lack of regeneration in these rats. In IFP-R rats (n = 5), 303 ± 91 CTB–Alexa 555–labeled neurons were found, respectively, in the right hypoglossal and facial nuclei 4 months after HN-FN neurorrhaphy, demonstrating successful double innervation of the whisker pad by both facial and hypoglossal axons (Fig. 4). Consistent with a previous study36 and with a partial preservation of hypoglossal functions, 177 ± 30 DY-stained neurons were observed in the hypoglossal nucleus (n = 5). Interestingly, we also found 14 ± 5 DY-labeled neurons in the facial nucleus, suggesting that a small number of regenerated facial axons may have innervated the ipsilateral hemitongue.

**Fig. 3.** Muscle action potentials were recorded from the right whisker pad during direct electrostimulation of the FN. Surface of MAPs are expressed in millivolts times milliseconds (mVmsec) and were measured prior to injury, 1 week after injury, 9 weeks after injury prior to repair surgery, 4 months after repair surgery before cutting the FN or the PNG, and 1 week after the cutting of either the FN or PNG. Results are presented as mean ± SEM and were analyzed by 2-way ANOVA: (time x treatment) [overall effect F(12, 74) = 6.67, p < 0.0001; effect of time F(4, 74) = 270, p < 0.0001; and effect of treatment F(3, 74) = 21.7, p < 0.0001]. Post hoc multiple comparisons between groups (n = 4–6) were conducted by Newman-Keuls tests. **p < 0.01 compared to groups with mean values at or below the dotted line.
Axonal Regrowth in PNGs and FNs

The presence of myelinated nerve fibers was further analyzed on semi-thin thionin-stained sections of the FN distal to the neurorrhaphy site and of the PNG. In intact rats (n = 4), we observed 3026 ± 141 myelinated axons within the FN (Fig. 5A). In contrast, at the end of the follow-up period, only aspects of degeneration were observed in the FN of CFP rats (n = 4, Fig. 5B). In IFP rats (n = 4), 856 ± 172 myelinated axons were counted in the FN, consistent with the aforementioned observation of partial spontaneous recovery of facial symmetry, nerve conduction, and target muscle reinnervation (Fig. 5C). Importantly, as many as 1472 ± 316 myelinated axons were observed in the FN of IFP-R rats 4 months after HN-FN “side”-to-side neurorrhaphy (n = 4, p < 0.001 [Fig. 5D]). In these IFR-R rats, about 677 ± 84 myelinated nerve fibers crossing the PNG were also observed (Fig. 5E), suggesting that they contributed to nearly one-half of...
the fibers reinnervating the right whisker pad after repair surgery (Fig. 5F). The presence of both myelinated and nonmyelinated axons in the FN and PNG of IFP-R rats 4 months after repair surgery and their absence in CFP rats was confirmed by electron microscopy (Fig. 6).

**Discussion**

Progress in microsurgical techniques now allows anatomical preservation of the FN in most cases during cerebellopontine angle surgery. For treating facial palsy after the FN injury, repair surgery is usually only performed in the absence of spontaneous recovery, requiring an important delay, often more than 1.5 years after the trauma.\(^8\)\(^,\)\(^32\) Unfortunately, such a long waiting period may result in an important and sometimes irreversible atrophy of the paralyzed facial muscles.\(^8\)\(^,\)\(^13\) Considering the clinical relevance, we developed a rat model of persistent IFP, and we explored a new “side”-to-side neurorrhaphy procedure for preserving the remnant facial axons and/or potential spontaneous regeneration. With such a surgical procedure, there would also be no more need for delaying repair surgery. On the other hand, the early innervation by the HN can prevent the occurrence of irreversible atrophy of the paralyzed facial muscles before spontaneous reinnervation, which has been referred to as the “baby-sitter” effect.\(^27\)
Experimental studies have shown that axonal regrowth occurs across a supercharged end-to-side\(^4,30\) or side-to-side\(^23,37\) nerve neurorrhaphy. Although this type of neurorrhaphy remains an area of intense scrutiny,\(^10,17,20,35\) many studies stress that such a nerve repair relies on injury to the donor nerve.\(^17,24,28,33\) Therefore, it is likely that injury to the donor nerve not only determines axonal regeneration from the donor to recipient nerve but also the absolute number of regenerated axons. Because the number of axons that effectively regenerate and the speed of elongation toward their targets constitutes the main factors that influence functional reinnervation,\(^12,18\) the cutting of one-half of the HN is probably necessary to provide an adequate source of motor axons. It has indeed been shown that in humans the cross-sectioned area and the number of myelinated axons of the normal FN account for about 61.5% of the area and 73.2% of the axon number in the normal HN.\(^2\) As expected, bridging one-half of the HN and the remnant axons of the injured FN with a PNG resulted in the double innervation of the paralyzed whisker pad by hypoglossal and facial motor neurons, leading to improved functional recovery. Successful double innervation was evidenced by the MAPs, the retrograde labeling of muscle innervation, and the analysis of regenerated facial axons and HN fibers growing through the PNG. Importantly, “side”-to-side neurorrhaphy with a PNG did not impair the regenerative capacity of spared facial axons, as approximately the same number of retrogradely labeled neurons was observed in the facial nuclei of IFP and IFP-R rats after injection of CTB–Alexa 555 into the whisker pad. However, the additional innervation of whisker pad muscles by hypoglossal axons regenerating through the PNG significantly improved functional outcomes. It is difficult to correlate the improvement of facial symmetry in rats by measuring the Angle \(\alpha\) to grades of the House-Brackmann system in humans. In the present study, we measured the Angle \(\alpha\) between a line extending from the fold on the bridge of the nose and a line linking the outer corners of each of the eyes, and found that \(\alpha\) was about 90° in intact rats and 71° in controls with complete facial paralysis. Although it is possible to create a scale system in rats based on Angle \(\alpha\) measurements, it may not reflect outcome evaluation in humans.

By sectioning either the PNG or the FN in IFP-R and IFP rats at the end of follow-up period, we also investigated which nerve pathway contributed to the recovery of facial symmetry. In IFP rats, sectioning of the FN resulted in the alteration of Angle \(\alpha\), which became comparable to that of CFP rats, and the loss of MAPs, indicating spontaneous functional reinnervation of whisker pad muscles by facial axons. In IFP-R rats, cutting either the PNG or the FN proximal to the neurorrhaphy site led to a deterioration of facial symmetry and a decrease in MAPs, demonstrating that both axons deviated from the hypoglossal and spared facial axons contributed to successful functional reinnervation. We assume that even more functional benefits may be obtained if the regeneration of hypoglossal axons through the PNG into the FN is further promoted, such as could occur using a PNG expressing NT-3 cDNA through lentiviral vector transduction.\(^36\)

In the present study, we performed nerve crush injury by sectioning all of the FN axons except the nerve’s perineurium and then ligated the injury site with 4-0 nylon sutures, which resulted in IFP after the spontaneous regeneration of part of the injured FN fibers. This method was assessed in our preliminary and present studies, in which the FN injury was standardized and nearly 20%–30% of the facial motor neurons spontaneously regenerated and reinnervated the FN, as evidenced by CTB–Alexa 555 retrograde labeling and MAP recording. However, in a clinical situation, it is difficult to precisely evaluate the FN damage, particularly for those patients whose FN is anatomically preserved. Progress in microsurgical techniques now...
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allows us to anatomicly preserve the FN in most cases during cerebellopontine angle surgery. Therefore, one of our objectives was to also test HN-FN “side”-to-side neurorrhaphy. Because the “side”-to-side neurorrhaphy at the injured FN is achieved through a window where only the epineurium is removed, this intervention does not interrupt the main structure of the FN at the neurorrhaphy site, thereby retaining the remnants of facial axons and the potential for spontaneous reinnervation. We hope this method will also be of interest for the treatment of other FN injuries, such as ischemia, stretch, thermal, and so on.

Surgical creation of new neural networks using end-to-side neurorrhaphy between a donor and a recipient nerve can give rise to synkinesis. However, we did not find any evidence of synkinesis in our IFP-R rats. This is consistent with the observation that the incidence of synkinesis is reduced after facial rehabilitation surgery if facial muscles are not only innervated by transposed hypoglossal axons but also by remnant facial axons.14 Hypoglossal neurons project axons to the facial nucleus at the level of the brainstem, and neurons of the parvocellular reticular nucleus innervate both facial and hypoglossal nuclei. These connections may explain coordinated movements of the tongue and facial muscles while swallowing and vocalizing.9,14 The shared innervation of facial and hypoglossal nuclei within the brainstem may prevent synkinesis of the doubly innervated facial muscles.1,7 Efficient facial network reconstruction should thus take into account existing connections within the brain. We assume that a conscious use of facial muscles may be regained after appropriate training during rehabilitation.11,29

Conclusions

This study shows that HN-FN “side”-to-side neurorrhaphy through a PNG can effectively treat persistent IFP in adult rats. The double innervation of the paralyzed whisker pad by such a neurorrhaphy could lead to rapid functional benefits without compromising the remnants of the facial axons, suggesting its potential application to treat persistent IFP in humans.

Disclosure

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