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# Experimental studies on post-transectional facial nerve regrowth and functional recovery of paralyzed muscles of the face in rats and mice

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**Abstract**

Insufficient recovery after peripheral nerve injury has been attributed to *(i)* lack of axonal navigation and poor pathfinding of regrowing axons to improper targets, *(ii)* excessive collateral axonal branching at the lesion site and *(iii)* polyneuronal reinnervation of the neuromuscular junctions (NMJ). The facial nerve transection model in rat and mice has been used to measure the restoration of function after varying therapies and to examine the mechanisms underlying their effects. Since it is still very difficult to combat the first reason and control/correct the navigation of several thousand axons, several groups of scientists concentrated our efforts on the postlesional branching (extremely strong in adult rats) and NMJ-polyinnervation. Since polyneuronal innervation of muscle fibers is activity-dependent attempts to reduce it were performed applying electrical stimulation. Unfortunately, the highly recommended intraoperative electrical stimulation of the proximal nerve fragment (square 0.1 ms pulses at 20 Hz using suprathreshold amplitudes) prior to suture and the postoperative electrical stimulation (square 0.1 ms pulses at 5 Hz) not only did not improve functional outcome, but reduced the number of innervated NMJ to approximately one fifth of normal values. Finally, recent experiments demonstrated that it was the mechanical (but not electrical) stimulation of denervated facial muscles (vibrissal and orbicularis oculi) which improved motor performance. This beneficial effect of mechanical stimulation was also detected after hypoglossal-facial anastomosis and inter-positional nerve grafting. The beneficial effect of manual stimulation on target muscle reinnervation was not present in mice deficient in the expression of insulin-like growth factor 1 and also eliminated or even reversed when trigeminal afferent inputs were abolished. All these findings raise hopes that clinically feasible and effective therapies could be soon designed and tested.

**Key words:** facial nerve; axotomy; facial palsy; motor endplates; polyinnervation; physiotherapy; electrical stimulation; mechanical stimulation

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## Introduction

The facial nerve is the most frequently damaged nerve in head and neck traumata. Apart from traffic-accident injuries (temporal bone fractures, or lacerations of the face), most facial nerve lesions are post-operative (removal of cerebellopontine-angle tumors, parotid resections because of malignancy). Despite the use of fine microsurgical techniques for repair of interrupted nerves in man, the recovery of voluntary movements of all 42 facial muscles, and emotional expression of the face remains poor.[1-4] The inevitable "post-paralytic syndrome", including mass movements (synkinesia) and altered blink reflexes,[5-7] has been attributed to *(i)* "misdi-

rected" reinnervation,[8,9] *(ii)* trans-axonal exchange of abnormally intensive nerve impulses between axons from adjacent fascicles,[10] and *(iii)* alterations in synaptic input to facial motoneurons.[11-13]

The misdirected or "aberrant" reinnervation has been recognized as the major reason for the post-paralytic syndrome. At the site of injury it has two components: *(i)* perhaps due to an insufficient and/or malfunctioning axonal guidance, a muscle receives reinnervation by "alien" axons, which have been misrouted along the "wrong" nerve fascicle;[14] *(ii)* due to the presence of competing supernumerary branches from all transected axons[15] one muscle fiber can be reinnervated by several motoneuronal axons (polyinnervation).[16,17]

Attempts to counteract with aberrant reinnervation, however had little success. So far it is technically impossible to steer properly the growth cones of several thousands axons growing out from the proximal stump of a transected nerve.[18,19] Likewise, efforts to reduce the degree of axonal branching in rats using artificial conduit as guiding scaffold have been unsuccessful: the process of axonal branching follows a rather constant pattern irrespective of local alterations of the extracellular matrix content.[20,21] Thus, although peripheral nerve injury is always followed by attempted regeneration of the injured axons, in everyday clinical practice, however, functional recovery is the exception rather than the rule.

In this review we summarize the efficiency of various therapies in rats by an extensive and combined methodological approach consisting of

*(i) biometrics of whisking behaviour* which provides a very sensitive tool to study the facial nerve regeneration.[22,23]

*(ii) successive pre- and post-operative retrograde fluorescent neuronal labeling to study the accuracy of target reinnervation*.[23-26]

*(iii) simultaneous multiple fluorescent neuronal labeling to quantitatively estimate the degree of axonal branching*.[20-22,27]

*(iv) combined immunostaining of axons (anti-neuronal class III β-tubulin) and histochemical staining of the neuromuscular junctions (AlexaFluor 488-conjugated α-bungarotoxin) to estimate the quality of target muscle reinnervation*.[28,29]

The results described in this review reflect our efforts to reduce collateral axonal branching by application to the nerve suture site of *(i)* neurite outgrowth fostering ECM proteins, *(ii)* factors causing local perturbation of microtubules' synthesis, *(iii)* suspensions of olfactory ensheathing cells (OEC), bone marrow-derived mesenchymal stem cells (BM-MSC) or Schwann cells (SC) and *(iv)* focal treatment with neutralizing antibodies to trophic factors.

Trying to reduce the intramuscular (terminal) axonal sprouting we proved the effect of *(i)* intraoperative electrical stimulation of the transected nerve, *(ii)* post-operative electrical stimulation of the denervated muscles, *(iii)* mechanical stimulation of the paralyzed mucles after facial-facial anastomosis (FFA), hypoglossal-facial anastomosis (HFA) and after interpositional nerve grafting (IPNG) of the facial nerve.

All manipulations influenced various parameters of peripheral nerve regeneration. However, only the application of manual mechanical stimulation of the paralyszed muscles yielded an improved recovery of vibrissae motor performance. Determining the mechanisms of action of the manual mechanical stimulation we found that this simple but very effective therapy requires intact afferent trigeminal input as well as insulin-like growth factor 1 (IGF-1).

## Generally Acknowledged Reasons for the Faulty Regrowth of Transected Peripheral Axons

Peripheral nerve injury initiates a complex series of changes. About 24 hours after disconnection, the axons in the distal fragment begin to lyse. When resorption of debris is complete, the Schwann cells re-arrange in the chains of Büngner[30] which bridge the interfragmentary gap and form guiding channels for the regenerating axons to their target(s). This so-called Wallerian degeneration creates an environment that is highly supportive for axonal re-growth and ensures that the vast majority of axons will enter the distal stump.[31] Nevertheless, complete recovery of function is only rarely achieved. Despite the use of modern microsurgical techniques for nerve repair, the aberrant re-innervation of motor targets, e.g. facial muscles, causes abnormally associated movements and altered reflexes.[3,5,7]

Minimal recovery has been attributed to misdirected (called also aberrant) regrowth of axons at the transection site and vigorous "intramuscular" or "terminal" sprouting of axons in the target muscles.[17]

At the site of injury, the aberrant reinnervation has two components. First, due to the malfunctioning axonal guidance, a muscle gets reinnervated by a "foreign" axon which has been simply misrouted along the "wrong" nerve fascicle.[14,32,33] Second, due to the presence of supernumerary branches from all transected axons ("collateral axonal branching"),[34-36] this given muscle can be reinnervated by branches stemming from several motoneurons, a state known as "polyneuronal innervation"[37,38] or "hyperinnervation".[39] Though claimed to be transient,[40] this aberrant innervation may persist for extended periods[41,42] with deleterious effects on synchronized function.

In the target muscles, regenerating axons branch at their terminals[43-49] such that the majority of motor endplates become poly-, rather than mono-, innervated.[28]

Whereas numerous aspects of the post-transectional aberrant reinnervation have been extensively documented,[50] little is known how this phenomenon could be prevented. Attempts to act on the first component of the aberrant reinnervation, achieving a "fascicular" or "topographic" specificity",[18,19] have failed: so far, it is technically impossible to guide correctly the growth cones of several thousands of axons (and their branches) originating from the proximal stump of a transected nerve.

This is why, most of the basic research laboratories concentrated their efforts on the reduction of the postlesional collateral axonal branching and on diminution of the neuromuscular junctions (NMJ) polyinnervation.

## Anatomy of the Infratemporal Portion of the Facial Nerve in Rats and Mice

The rat facial nucleus resides in the pons/rostral medulla. It contains 4500-5500 multipolar motoneurons grouped in 5 subnuclei: lateral, dorsal, intermediate, medial and ventromedial[51-53] with no apparent differences in the diameter of the perikarya (35-40 µm).[54]

As in humans, fibers course dorsomedially around the abducens nucleus. Then they leave the ventrolateral surface of the medulla ventrally to the cranial nerve VIII. The mean diameter of the myelinated axons in the facial nerve is 2.75 ± 0.77 µm.[54]

The facial nerve then enters the temporal bone (pars petrosa) through the internal acoustic meatus dorsal to the vestibulocochlear nerve. It emerges from the skull through the stylomastoid foramen to innervate the muscles of expression.[55,56]

The first branches of the free portion of the facial nerve are the internal and caudal auricular nerves, which supply the muscles of the ear, some hyoid muscles and the caudal belly of the digastric muscle.[57] The main trunk enters the face by turning around the mandible where it lies between the masseter muscle and the parotid gland.[55] It then divides into three branches, the zygomatic, the buccal and the marginal mandibular branch **(Figure 1).**

## Application of Extracellular Matrix (ECM) Proteins to Reduce Collateral Branching of Axons at the Lesion Site Turns out to be Unsuccessful

Injury to the peripheral nerve sets initiates a complex series of changes distal to the site of injury, collectively known as Wallerian degeneration. Within 24 hours after lesion, the axonal content begins to necrotize and axonal debris is phagocytosed by blood-born macrophages and proliferated Schwann cells.[58-60] When resorption is complete, the Schwann cells form long chains of cells (bands of Büngner), which bridge the interfragmentary gap and form guiding channels for the regenerating branches on their way to the target(s). The architectural pattern of the Büngner's bands of the peripheral stump remains unchanged for 3 months, after which progressive distorsion by proliferating connective tissue occurs. The process of Wallerian degeneration creates an environment that is highly supportive for axonal growth. The preference for axonal growth into a degenerating nerve ensures that the vast majority of axons will regrow into the distal stump if it remains in continuity with the proximal stump.[31]

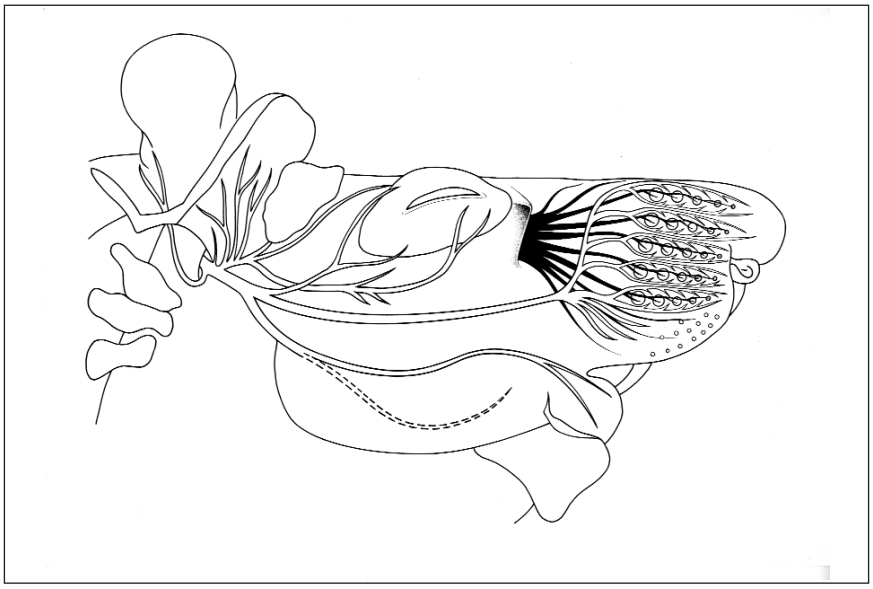

**Figure 1.** Schematic drawing of the infratemporal rat facial nerve *(plexus parotideus)* illustrating the close relationship between the infraorbital nerve (in **black**) and the buccal branch of the facial nerve. Adapted from Semba and Egger[53] and Dörfl.[56]

In spite of that, the regenerating axons do not merely elongate towards the distal stump, but respond with axonal branching (sprouting) by lateral budding mainly at the nodes of Ranvier, up to 6 mm proximal to the injury site. As regeneration proceeds, some of these supernumerary branches are pruned off over a period of up to 12 months.[61] There are, however, persistently higher numbers of myelinated and unmyelinated axons in regenerated segments of peripheral nerves than in intact nerves.

What is the general biological significance of branching? To answer this question, one needs more information about the structural and biochemical events which accompany the process of axonal sprouting. The majority of the recent reports suggest that the axonal branching is part of the neuronal response to injury within a complex program directed towards regeneration. This attempt is associated with substantial cytoskeletal reorganization,[62] resulting in the elaboration of fine protrusions (sprouts) into and across lesion sites.[63]

Observations *in vitro* show that axonal branching begins from the end-bulb within 3 hours after injury.[64] The regenerating branches initially lie on the surface of the Schwann cells. Later, these branches increase in diameter and get surrounded by Schwann cell processes. The guidance of these immature axons to their final destination can be considered as a series of short-range projections to intermediate targets under the influence of local guidance cues. Neurons respond to these cues by means of motile sensory apparatus at the tip of the advancing axon termed the "growth cone", which very often does not emerge from the axon at the precise site of injury, but proximal to it. The initial formation of growth cones occurs before the necessary newly synthesized proteins would have time to arrive at the site of axon injury, i.e. too rapidly to be dependent on metabolic changes in the cell body.[65,66]

The growth cone borne by neurites is shaped like a webbed foot.[67] There is a swollen central core from which flattened processes called lamellipodia and numerous stiff fine processes called filopodia extend. Current studies have identified 3 major intracellular cytoskeletal components responsible for the cytomechanical forces in the leading edge of elongating axons: actin microfilaments, myosin and microtubules.[68]

Based on reports that the peroral administration of drugs, which accelerate axonal regrowth, synchronously reduces the postoperative hyperinnervation of muscles[39,69] several scientific groups tested, whether fostering axonal regrowth by the local application of collagen type I,[70] fibronectin, laminin[42,71-73] or tenascin-R[74,75] might prevent the occurrence of supernumerary axonal branches and thus also improve target reinnervation.

Dohm et al.[20] and Hristov et al.[76] performed entubulations of transected rat facial nerve in a chamber **(Figure 2)** filled with phosphate buffered saline (PBS) pH 7.4, collagen type I (100 µg/ml in PBS), laminin (20 µg/ml in collagen type I), fibronectin (20 µg/ml in collagen type I), tenascin-R (20 µg/ml in collagen type I), semaphorin 3A/Fc chimera (120 ng/ml in collagen type I), neuropilin-1/Fc chimera (3 µg/ml in collagen type I). Two months later, they performed a complex analysis which was based on three main separate investigations.

## Video-based motion analysis of vibrissae motor performance

First they examined the effects of these molecules on the return of function. Video analysis of vibrissal motion allowed a detailed assessment of vibrissal whisking.[23,25] Normal animals explore the environment by coordinated sweeps of individual vibrissae ("whisking") with a frequency of about 6-7 Hz.[77-80] Vibrissal movements are characterized by an active protraction rostrally via muscle contraction and as previously described, by an active retraction caudally.[81] The amplitude of the movement from maximum protraction to maximum retraction was about 50º **(Figure 3).** Compared to intact animals, vibrissal motion was very poor in the rats of all groups: the amplitude was reduced in average by 70% and angular velocity by 80%.

## Estimation the degree of collateral branching of axons at the lesion site

Next, triple retrograde labeling was used to assess the projection patterns of motor axons from the facial nucleus through its different motor rami as well as the degree of axonal branching.[20] In intact animals, motoneurons with axons entering the zygomatic, buccal or marginal mandibular rami **(Figure 4a)** are localized in the dorsal, lateral and intermediate facial subnuclei, respectively.[53] No double- or triple-labeled motoneurons are observed because intact motoneurons send only one unbranched axon to one of the facial nerve rami **(Figure 4b).** Thus, the index of axonal branching in the facial nerve trunk of intact animals, calculated from the zygomatic motoneurons, is 0%.

Two months after facial nerve cut and suture **(Figure 4c)** or cut and entubulation with various ECM-proteins **(Figure 4e),** myotopic organization into subnuclei was no longer observed, i.e. all retrogradely labeled motoneurons were scattered throughout the facial nucleus **(Figures 4d** and **f).** This lack of myotopy was presumably due to the numerous collateral branches[34] emerging from individual transected axons which per-

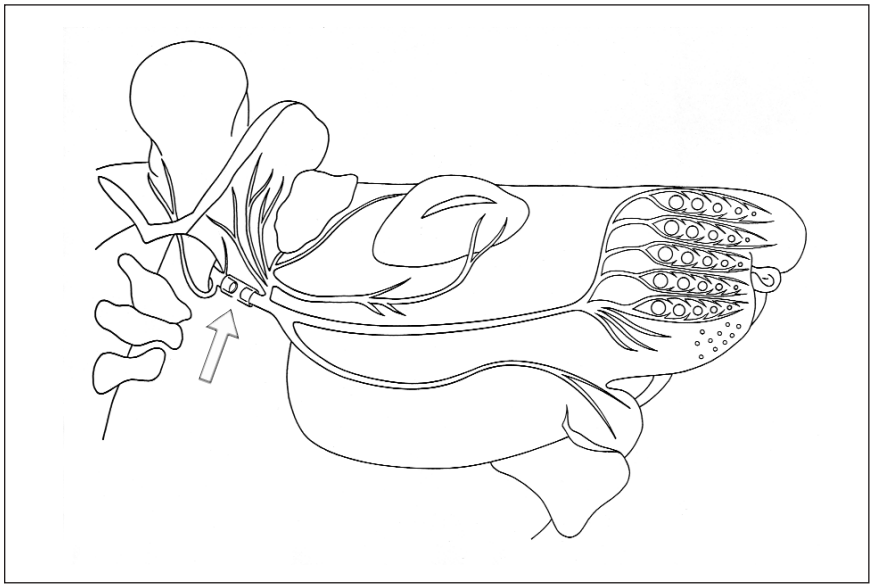

**Figure 2.** Schematic drawing illustrating the exact entubulation site of the facial nerve (arrow). Adopted from Dohm et al.[20]

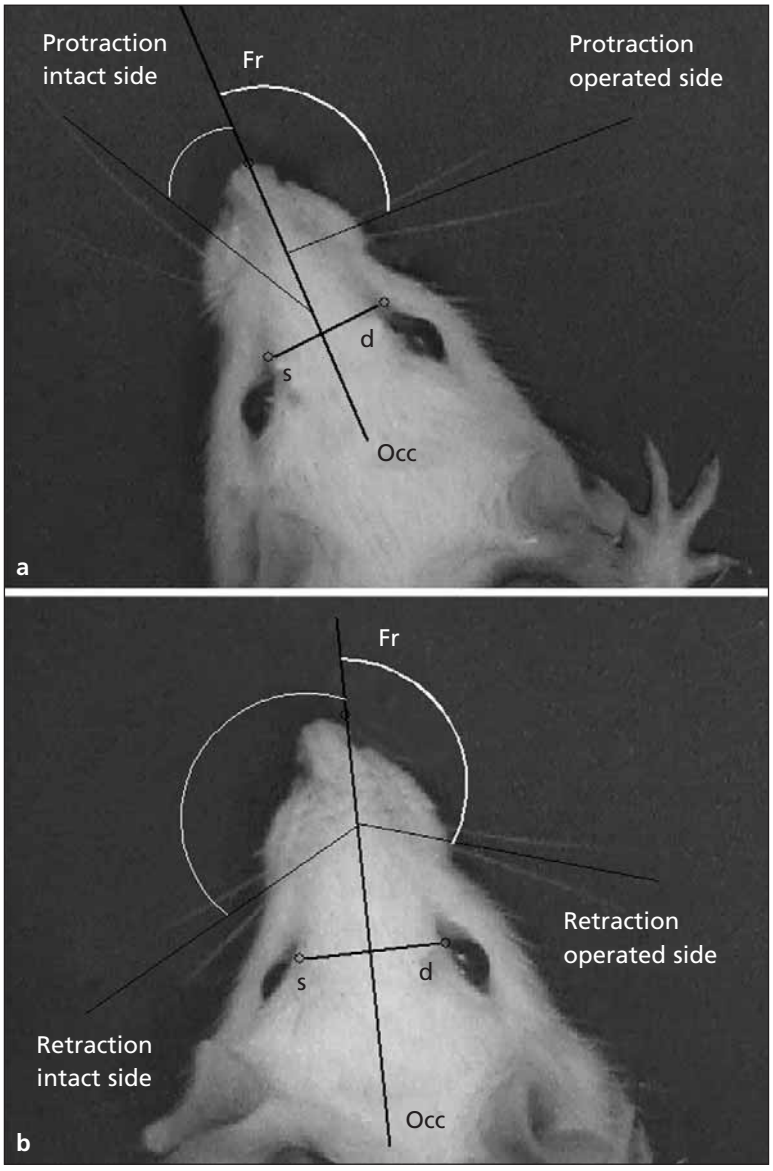


**Figure 3.** Video-based motion analysis of vibrissae motor performance. Angles, angular velocity, and angular acceleration on the intact (*left*) and operated side (*right*) were measured during vibrissal protraction (**a**) and retraction (**b**). Note the significant change in angle from the sagittal line (**Fr-Occ**) during protraction and retraction on the intact side; vibrissae on the operated side remain spastic. **Fr:** frontal; **Occ:** occipital; **d:** dexter; **s:** sinister. Adopted from Tomov et al.[23]

sisted, grew into different rami and retrogradely transported the different fluorescent dyes to their parent motoneurons in the facial nucleus.

In addition, double and triple labeling of motoneuronal perikarya was commonly observed **(Figures 4d** and **f).** The only explanation for multiple labeling is that the axonal branches projected into different facial nerve rami (e.g. zygomaticus, buccalis or marginalis mandibulae) and therefore retorgradely transported two or three fluorescent dyes simultaneously to the parent perikarya. Finally, as a result of this collateral branching at the lesion site, each of the individual facial nerve rami contained axons or axonal branches of more motoneurons than in intact animals, which in the periphery caused hyperinnervation of targets.[38,39,82]

In this experimental set, the index of axonal branching was 50-70% (sum of the percentages of all retrogradely double-labeled facial perikarya). None of the therapeutic entubulations had a significant influence on the projection patterns. Thus, there was a complete lack of myotopic organization, increased total numbers of projecting motoneurons and a consistently elevated

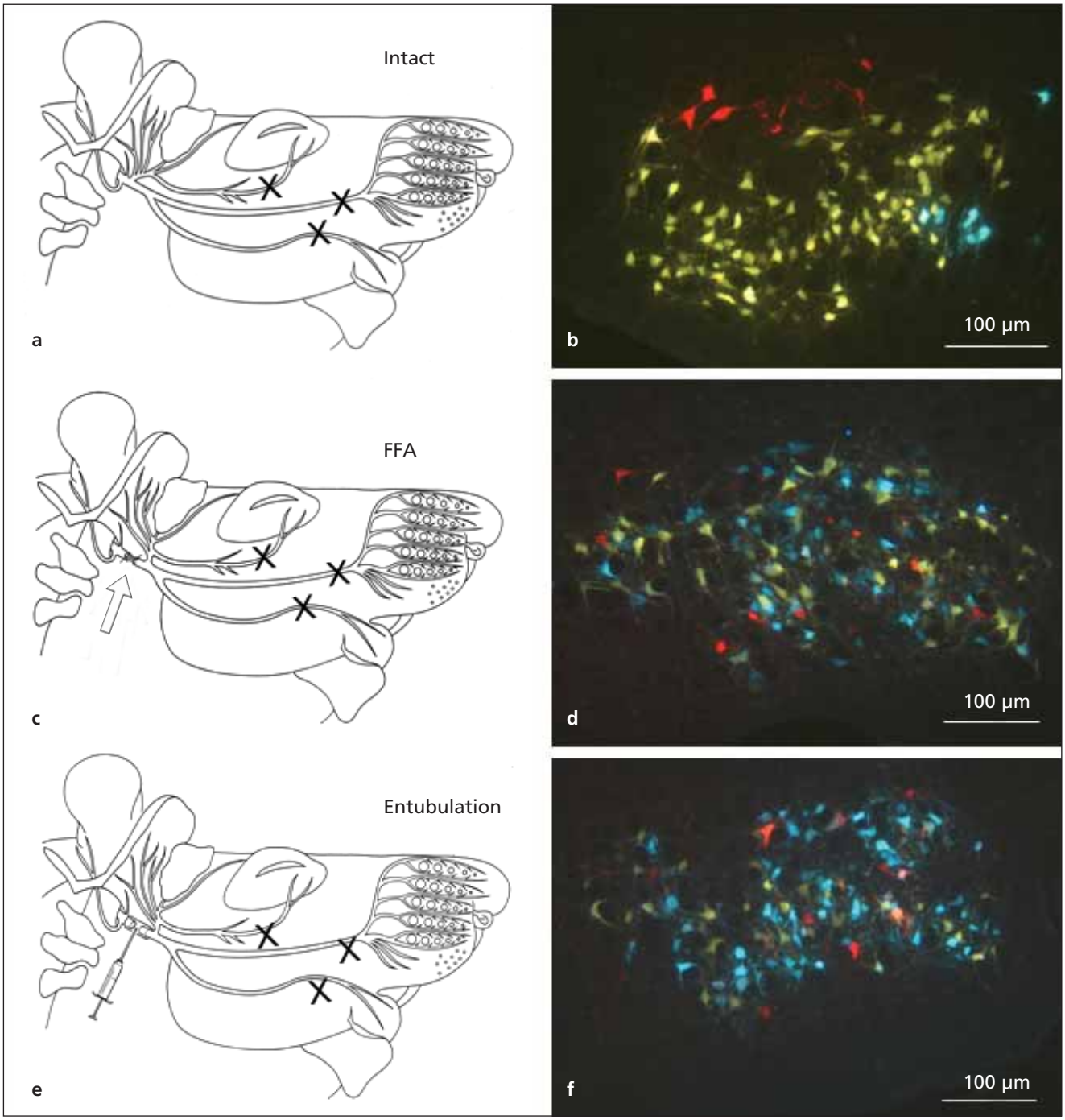


**Figure 4.** Myotopic organization of the facial nucleus and collateral axonal branching as estimated by the pattern of retrograde labeling. In **intact** animals (**a** and **b**), simultaneous application of DiI (**red**), FG (**yellow**) and FB (**blue**) to the zygomatic, buccal and mandibular nerve branches respectively labels distinct subnuclei with no overlap. Two months after transection and suture (**FFA**) (**c** and **d**) or **entubulation** (**e** and **f**) the myotopic organization is lost and there occurred numerous double labeled motoneuronal perikarya. Adopted from Streppel et al.[21]

degree of axonal branching regardless of whether the animals were subjected to any of the therapeutical paradigms or not. Obviously, the branching capacity of lesioned motoneurons is so strongly determined that even significant changes in the local microenvironment of the lesion site are not able to suppress it.

### Estimation the degree of polyinnervation of the NMJ

Finally, the re-innervation pattern of individual skeletal muscle fibers was investigated **(Figure 5a).** Although post-lesional polyinnervation of the end-plates has been claimed to be transient,[40] accumulating evidence suggests that it persists after establishment of nerve-muscle contacts[32,41,42,83-87] and previous work indicates that it has a deleterious effect on recovery of facial motor function.[28] In intact animals, all motor endplates of the largest vibrissal muscle, the levator labii superioris, were monoinnervated **(Figure 5c).** After facial nerve transection and suture, 53% were polyinnervated, i.e. innervated by two or more axons **(Figure 5b).** Neither entubulation reduced the proportion of polyinnervated endplates (about 50%).

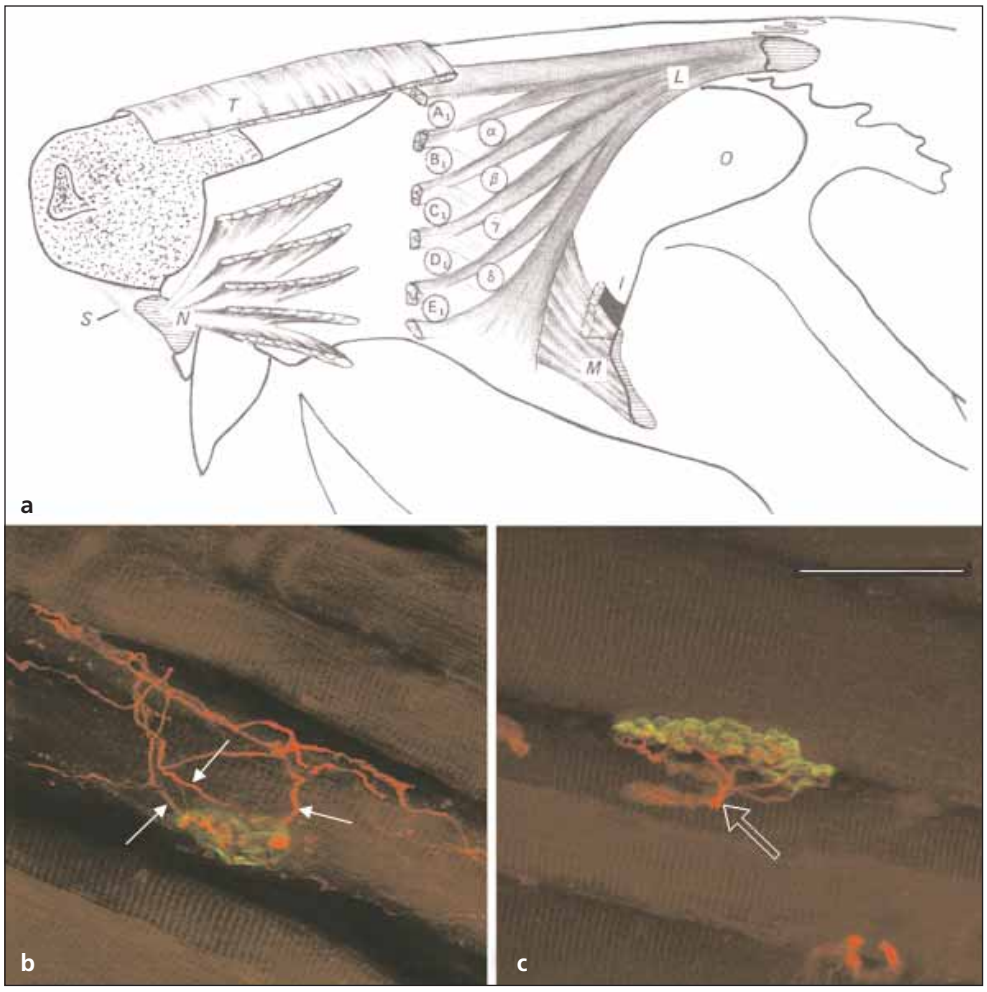


**Figure 5.** Quality of target muscle reinnervation. **a:** Schematic drawing of the extrinsic vibrissae muscles. **α-δ**: the four caudal hair follicles, the muscles slings of which "straddle" the five vibrissae rows (**A-E**); **I:** infraorbital nerve **L:** m. levator labii superioris; **M:** m. maxillolabialis; **N:** m. nasalis; **O:** orbit; **S:** septum intermusculare; **T:** m. transversus nasi. **b** and **c:** Superimposed stacks of confocal images of end-plates in the levator labii superioris muscles of intact and surgically treated rats visualized by staining of the motor end-plates with Alexa Fluor 488 α-bungarotoxin (*green fluorescence*) and immunostaining of the intramuscular axons for neuronal class III β-tubulin (Cy3 *red fluorescence*). Panels **b** and **c** show examples of polyinnervated and a monoinnervated end-plate, respectively. Three axonal branches (**arrows** in **b**) reach the boundaries of the polyinnervated end-plate delineated by the alpha-bungarotoxin staining. In contrast, the monoinnervated end-plate is reached by a single axon (**empty arrow** in **c**) with several preterminal rami. In both examples, the whole end-plates are within the stack of confocal images. Scale bar shown in **c** indicates 125 μm. Adopted from Grosheva et al.[145]

## Local Perturbation of Microtubules' Synthesis Reduces Collateral Branching of Axons at the Lesion Site and Improves Recovery of Whisking Function

Accumulating knowledge shows that neurite regrowth is part of the neurons' receptor-mediated response to extracellular guidance cues.[88] Since most receptor-mediated signal transduction pathways converge on to the Rho-family of small GTPases, axonal elongation is associated with substantial reorganization of the cytoskeleton.[62,63,89,90] Accordingly, an alteration of the dynamics of postlesional cytoskeletal reorganization may lead to an increased rate of axonal regrowth. There are 3 major intracellular cytoskeletal components responsible for the cytomechanical forces in the leading edge of the axon: actin microfilaments, myosin and microtubules.[68] In their recent work, Peeva et al.[91] concentrated our observations on the microtubules.

A microtubule is a long, hollow cylinder that is made of a polymer of α- and β-tubulins and has a diameter of 25 nm. It has intrinsic polarity, with a fast-growing 'plus end' and an opposite, slow-growing 'minus end'. In axons, microtubules run in a longitudinal orientation and serve as rails along which membranous organelles and macromolecular complexes can be transported; they are unipolar, with the plus end pointing away from the cell body.[92]

Facilitating the fusion of vesicles with the plasma membrane, microtubules have been shown to promote the extension of growth cone lamellipodia.[93,94] Upregulated levels of tubulin in the perikarya and increased delivery of mictotubules to regrowing axon tips have been considered essential for regeneration.[95-98] Accordingly, Schaefer et al.[99] and Fukata et al.[100] have shown that the population of microtubules that invade the peripheral domain via filopodia are highly dynamic, suggesting functional specializations, perhaps in exploratory and/or signaling capacity.

Finally, drugs that attenuate either microtubule or actin dynamics (inhibition of actin polymerization with cytochalasin, stabilization of microtubules with taxol, or damping of microtubule dynamics with vinblastine) have been shown to inhibit *in vitro* axonal branching but not elongation.[101-104] Treatment with vincristine, an inhibitor of microtubule formation blocks the outgrowth of some axons and delays the regeneration of others.[105]

As a logical continuation of these *in vitro* studies, Grosheva et al.[106] proved whether a similar treatment *in vivo* would also increase the rate of neurite regrowth and improve recovery of muscle function. Such a test was not only highly relevant to everyday clinical practice, but also could be applied very rapidly – some pharmacological agents that affect microtubule dynamics are registered and established drugs for use in human patients.

In this work Grosheva et al.[106] applied established pharmacological agents to perturb microtubule assembly towards stabilization (enhanced polymerization with 10 µg/ml taxol) or increased synthesis (challenged by destabilization with 100 µg/ml nocodazole and 20 µg/ml vinblastine) to the transected buccal branch of the rat facial nerve.

Evaluation of the effect(s) two months later included estimations of *(i)* vibrissae motor performance by video-based motion analysis, *(ii)* degree of collateral axonal branching by double retrograde neuronal labeling with crystals of Fluoro-Gold and DiI and *(iii)* pattern of motor end-plate re-innervation (proportions of mono- and poly-reinnervated) in the largest extrinsic vibrissal muscle, the levator labii superioris. They found that only stabilization of microtubules with 10 µg/ml taxol reduced intramuscular axonal sprouting and polyinnervation of the motor end-plates which was accompanied by improved restoration of function.[106]

## Local Application of Olfactory Ensheathing Cells (OECs), Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSC) or Schwann Cells (SC) to Reduce Collateral Branching of Axons at the Lesion Site does not Improve Quality of Reinnervation and Recovery of Whisking Function

The failure of ECM proteins to foster rapid axonal regrowth and thus reduce collateral axonal branching [*see* Chapter: Application of Extracellular Matrix (ECM)

Proteins to Reduce Collateral Branching of Axons at the Lesion Site Turns out to be Unsuccessful] suggested that the amount of neurotrophic factors at the lesion site may be unsufficent. In order to test this hypothesis, several scientific groups decided to transplant dissociated glial cells into the lesion site and prove whether they might act as a trophic-factor source. In addition, glial cell transplantations also aimed at reconstructing the nerve connection by filling a large gap between the proximal and distal nerve stump with cell suspensions applied within guidance channels.

### Schwann cells (SC) and olfactory ensheathing cells (OECs)

They have been used since they are closely-related glial cell types that commonly express a number of neurotrophic molecules,[42,107,108] e.g. the brain-derived neurotrophic factor (BDNF) and the ciliary neurotrophic factor (CNTF). These molecules have been shown to affect neurite outgrowth and sprouting.[109-112] Furthermore OECs have been shown to stimulate regeneration of central and peripheral neurons.[113-116] Comparing the effects of glial cell transplantation on CNS axon regrowth, it was found that SC increase sprouting at the lesion site whereas OECs reduce sprouting but increase growth into the distal part of the lesioned spinal cord.[115,117-119]

### Bone marrow-derived mesenchymal stem cells (BM-MSC)

Stem cells are undifferentiated cells that renew themselves to maintain the stem cell pool. At the single-cell level they should be able to differentiate into more than one mature, functional cell. In addition, when transplanted, stem cells should be capable of replacing a damaged organ or tissue for the lifetime of the recipient.[120] BM-MSC are pluripotent[121] and have neuronal[122] and glial[123,124] differentiation potential.

BM-MSC can differentiate along a glial or Schwann cells lineage[125-135] and have therefore been considered as potential candidates for improving the rate of axonal regrowth and re-myelination by reducing the degree of axonal branching[136,137] and hopefully, therefore, improve function. And indeed, transplanted BM-MSC have been shown to improve recovery of function after injury of rat sciatic nerve[138-142] and to remyelinate the spinal cord.[143,144]

Compared to intact animals, vibrissal motion was poor in rats receiving any entubulation of the facial nerve. The amplitude was reduced respectively by an average of 70% and angular velocity by 80%. Due to poor axonal pathfinding and excessive growth of axonal branches at the lesion site, there was no myotopical organization of the facial nucleus into subnuclei and the index of collateral branching was about 70%. Neither entubulation reduced the proportion of polyinnervated endplates.[22,145,146]

## Focal Treatment with Neutralizing Antibodies Against the Soluble Neurotrophic Factors NGF, BDNF, FGF-2, IGF-I, CNTF, GDNF Reduces Collateral Axonal Branching at the Lesion Site, but Promotes no Recovery of Whisking Function

Based on our results showing that, in spite of their known effect to support neurite elongation, neither ECM proteins, nor cultured OECs, BM-MSC, or SC suppressed the redundant axonal branching,[20,22,145,146] Streppel et al.[21] hypothesized that that the aberrant axonal branching could, at least in part, be due to the increased expression of trophic molecules at the lesion site. Accordingly, an inhibition or blockade of these factors should reduce branching and improve the accuracy of reinnervation. This is why they applied neutralizing antibodies to several neurotrophic agents and checked whether some of them would reduce the proportion of branched axons.

Following analysis of local protein expression by immunocytochemistry and by *in situ* hybridization, the facial nerve trunk of adult rats was transected and insert-

ed both ends into a silicon tube containing *(i)* collagen gel with neutralizing concentrations of antibodies to NGF, BDNF, bFGF, IGF-I, CNTF and GDNF; *(ii)* five-fold higher concentrations of the antibodies; *(iii)* combination of antibodies.

Two months later, retrograde labeling was used to estimate the portion of motoneurons the axons of which had branched and projected into 3 major branches of the facial trunk. After control entubulation in collagen gel containing non-immune mouse IgG, 85% of all motoneurons projecting along the zygomatic branch sprouted and sent at least one twin axon to the buccal and/or marginal-mandibular branches of the facial nerve. Neutralizing concentrations of anti-NGF, anti-BDNF and anti-IGF-I significantly reduced sprouting. The most pronounced effect was achieved after application of anti-BDNF, which reduced the portion of branched neurons to 18%. All effects after single application of antibodies were concentration-dependent and superior to those observed after combined treatment. Thus, treatment of rats with antibodies against NGF, BDNF, bFGF, IGF-I, CNTF or GDNF increased the precision of reinnervation, as evaluated by multiple retrograde labelling of motoneurons, more than two times as compared to control animals.[21]

The subsequent biometric analysis of vibrissae movements however, did not show positive effects on functional recovery suggesting that polyneuronal reinnervation of the motor end-plates- rather than collateral branching – may be the critical limiting factor. In support of this hypothesis, Guntinas-Lichius et al.[28] found that motor end-plates with morphological signs of multiple innervation were much more frequent in reinnervated muscles of rats which did not recover after injury (51% of all end-plates) compared to animals with good functional performance (10%).

This milestone report[28] provided, for the first time, controlled experimental evidence for the contribution of axonal branching and misdirection to the failure of recovery of function following facial nerve injury. By manipulating the local environment using neutralizing antibodies to growth factors, a strong reduction in collateral axonal branching from the proximal stump was achieved. In the same animals, however, function of the re-innervated vibrissae muscles remained as poor as in non-treated injured animals. As a potential reason for the ineffectiveness of the treatment Guntinas-Lichius et al.[28] identified the well-known post-transectional polyneuronal innervation of the motor endplates, a phenomenon which was not directly manipulated in our experiments. These results raised questions of fundamental importance with regard to the mechanisms limiting functional recovery and to the perspectives for identifying new efficient treatment strategies.

Furthermore, since polyneuronal innervation of muscle fibers is activity-dependent and can be manipulated, these findings raised hopes that clinically feasible and effective therapies could be soon designed and tested.

## Intra-operative Electrical Stimulation of the Proximal Stump before Nerve Suture Fails to Improve Quality of Target Reinnervation and Functional Recovery after Nerve Repair

Although clinicians and researchers have tested the usefulness of electric current applications for enhancement of peripheral nerve regeneration for more than a century, the efficacy of such treatment for facial palsy has remained questionable.[3,147] For example, clinical experience has shown positive effects on occasion[148,149] but, mostly, electrical stimulation has been considered to be inefficient.[150,151]

Recently, a novel clinically feasible approach to enhance peripheral nerve regeneration after femoral nerve (a mixed nerve i.e. sensory and motor) lesion in rats was suggested.[152-155] Brief, low-frequency electrical stimulation (1 hour, 20 Hz) is delivered to the proximal nerve stump of the severed nerve prior to its surgical reconstruction. Stimulation leads to depolarization of the motoneuron perikarya and a significant shortening of the period of asynchronous, "staggered" axonal

regrowth; in addition, preferential motor reinnervation is accelerated.[156,157] These beneficial effects are associated with a faster and enhanced up-regulation of brain-derived neurotrophic factor (BDNF) and its tyrosine kinase B (TrkB) receptor in motoneurons;[158,159] in addition, TrkB-dependent expression of the HNK-1 (human natural killer cell antigen-1) glycoepitope is increased in the quadriceps branch of the femoral nerve.[160] Brief electrical stimulation after sciatic nerve injury also promotes axonal regeneration and attenuates facilitation of spinal motor responses.[161]

Recent experiments have also shown that, although the brief stimulation protocol considerably accelerates functional recovery after femoral nerve repair in mice, it does not however improve the long term, final outcome.[162] This is why Skouras et al.[163] tested the therapeutic potential of the treatment in the facial nerve (purely motor) injury paradigm in rats, a regeneration model system differing in many aspects from the femoral nerve paradigm such as motoneuron connectivity, nerve composition and muscle properties.

Testing the efficacy of electrical stimulation in the facial nerve paradigm appeared especially warranted considering that most of the clinical experience, and controversy, associated with electrical stimulation is related to treatment of facial palsy.[164-166] Rates were subjected to one hour of electrical stimulation immediately following nerve cut and prior to nerve repair by end-to-end anastomosis **(Figure 6).** Video-based motion analysis was used to monitor vibrissal motor performance over a 4-month recovery period, a non-invasive approach allowing precise and longitudinal assessment of whisker pad muscle function.[23] Subsequently morphological analyses involving retrograde tracing and endplate morphology on the same animals were performed[21,28] to elucidate, respectively, whether ES influenced the degree of collateral axonal branching at the lesion site and/or motor end-plate polyinnervation, both of which are associated with functional deficits following facial nerve injury.

The results of Skouras et al.[163] showed that in adult rats, the brief electrical stimulation immediately after transection and for 1 hour prior to end-to-end suture of the severed facial nerve, a purely motor nerve tract, does not lead to improved motor recovery at 4 months. Regardless of whether the animals were electrically stimulated or not, the degree of collateral branching of axons at the lesion site was high (50-70%), the proportion of polyinnervated motor end-plates in the musculature was approximately 50% and the amplitude of vibrissal whisking remained at 25-30% of that in intact animals.

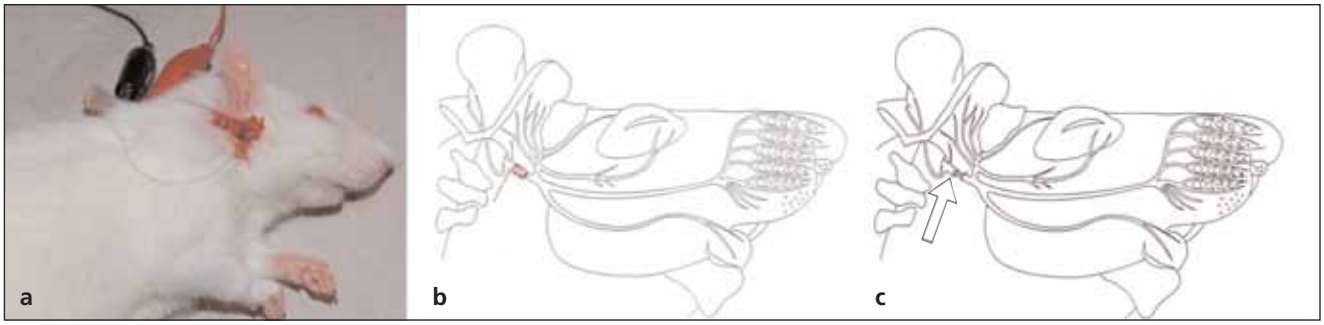


**Figure 6. a:** Intraoperative electrical stimulation of the proximal stump of the transected right facial nerve. **b:** The right facial nerve was exposed and a teflon-coated stainless steel wire (50 μm in diameter, bared of insulation at its tip) was twisted to form a loop around the nerve stump. **c:** A second electrode, used as an anode, was fixed to a muscle close to the nerve. In all electrically stimulated rats, the threshold voltage required to elicit visible contractions of the whisker pad muscles was determined by applying square 0.1 ms pulses at 20 Hz at varying voltage intensities using a pulse generator (Master-8, A.M.P.I., Jerusalem, Israel). Immediately thereafter, the nerve stump was transected with fine scissors about 2 mm distally from the electrode. The proximal nerve stump was then stimulated for 1 hour by applying square 0.1 ms pulses at 20 Hz using amplitudes 3 times above threshold levels (typically 3-4 V). Thereafter, the electrodes were removed, and the ends of the nerve were sutured with single epineural 11-0 nylon stitches (**arrow**). Adopted from Skouras et al.[163]

## Post-operative Electrical Stimulation of Paralyzed Vibrissal Muscles does not to Promote Motor Recovery After Facial Nerve Repair

Electrical stimulation (ES) of denervated muscles is a potent therapy which, maintaining muscle mass and structural integrity, can counteract loss of muscle excitability and muscle atrophy resulting from disuse.[167-171] However, evidence has yet to be presented as to whether, and to what degree, preservation of a larger muscle mass and better functional properties of denervated muscles would promote functional recovery after reinnervation.

To elucidate these issues further and provide evidence for the therapeutic benefit or otherwise of ES, Sinis et al.[172] examined facial nerve injury in the rat model focusing on whisking as the functional readout. Following facial nerve lesion, rats received ES or sham stimulation (SS) of the vibrissal muscles over a 2-month period at stimulation intensities sufficient to depolarize the regenerating intramuscular nerves **(Figure 7).** Starting on the first day after end-to-end suture (facial-facial anastomosis), ES (square 0.1 ms pulses at 5 Hz at an ex tempore established threshold amplitude of between 3.0 and 5.0 V) was delivered to the vibrissal muscles for 5 minutes a day 3 times a week.

Restoration of vibrissal motor performance following ES or SS was evaluated using video-based motion analysis and correlated with the degree of collateral axonal branching at the lesion site, the number of motor end-plates in the target musculature and the quality of their reinnervation, i.e. the degree of mono- versus poly-innervation. Neither protocol reduced collateral branching. ES did not improve functional outcome, reduced the number of innervated motor end-plates to approximately one fifth of normal values and failed to reduce the proportion of polyinnervated motor end-plates. It is concluded that ES is not beneficial for recovery of whisking function after facial nerve repair in rats.[172]

## Mechanical Stimulation of Paralyzed Vibrissal Muscles Improves Quality of Motor Target Reinnervation and Promotes Recovery of Whisking Following Facial Nerve Repair

Clinically, there are few options for treating denervated muscles. One possibility is electrical stimulation (ES), although a great deal of controversy surrounds its use with either some benefit[148,149,173,174] or no effect[151,164-166,175-178] being described. Electrical stimulation of denervated soleus muscle inhibits intramuscular sprouting and diminishes motor-end plate polyinnervation.[179,180] However, regular ES of totally denervated muscle fibers suppresses the production of chemical mediators required for reconnection of an axon branch with its motor endplate on the muscle and also reduces the spontaneous electrical activity of orphaned muscle fibers (fibrillation) which is thought to be a signal for sprouting of the remaining healthy motor nerve.[181,182] By contrast, ES


**Figure 7. a:** Schematic drawing of the infratemporal portion of the rat facial nerve. The site of transection and end-to-end suture of the facial nerve trunk, i.e. facial-facial anastomosis (FFA) is indicated by an **arrow. b:** Sham-stimulation of rats. Acupuncture needle electrodes were inserted, but no current was applied to the electrodes. **c:** Postoperative electrical stimulation of the vibrissal muscles. Adopted from Sinis et al.[172]

of muscle fibers that retain a partial nerve supply may simulate voluntary muscle overuse and contribute to suppression of the chemical mediators required for the reinnervation of the denervated fibers.[183] For the above reasons, ES is not a method of choice and indeed has not been widely used to treat facial paralysis.

Following denervation and before reinnervation, severe changes also occur within the muscle including loss of muscle bulk and circulation; connective tissue also shrinks and becomes adherent (fibrosis).[184-186] After several months of complete denervation, muscle membrane properties change, becoming relatively non-responsive to electrical stimulation.[187-189] For patients expected to have nerve re-growth after complete denervation, it is important to minimize fibrosis within the muscle connective tissue so that there will be movable muscle structures after muscle re-innervation to allow reacquisition of the contractile proteins that make muscles work.[190-193]

Angelov et al.[29] therefore decided to try a novel approach and use mechanical stimulation of facial nerve injury. Based on clinically established positive benefits of soft tissue massage, supposed to promote muscle blood flow and to keep in optimum condition whilst awaiting nerve recovery,[150,194-196] the vibrissae and whisker pads were gently stroked by hand for 5 minutes daily for two months after transection and suture of the facial nerve.

The first signs of vibrissal motor performance recovery were detected at 4-5 weeks post transection and within the following weeks, recovery reached its maximum and stayed at this level for the next two months. Whisker pads and vibrissae were therefore stroked gently by hand for 5 minutes daily for two months after transection and suture of the facial nerve. Mechanical stimulation was compared with *(i)* "environmental" stimulation whereby rats' vibrissae encountered objects in an enriched environment, *(ii)* holding animal in the same manner and for the same amount of time as used during manual stimulation (i.e. a sham "handling" control) and *(iii)* manual stimulation of the intact contralateral whiskerpad **(Figure 8).**

Only the manual ipsilteral stimulation resulted in a complete return of normal vibrissal motor performance with a concomitant pronounced reduction in polyinnervation.[29] This report provided the first controlled experimental evidence for the efficacy of mechanical muscle stimulation to improve functional recovery after facial nerve injury in the rat. By stroking the whiskers Angelov et al.[29] stimulated their fine vibrissal muscle slings innervated by the facial nerve, achieved a significantly reduction of the proportion of polyinnervated motor endplates and full recovery of vibrissal motor performance. These findings show that manual mechanical stimulation can contribute to improved recovery for facial nerve injuries[197] and that clinical studies may be warranted.

## Manual Stimulation of Facial Muscles Improves Functional Recovery after Hypoglossal-Facial Anastomosis and Interpositional Nerve Grafting of the Facial Nerve

Clinically, soft tissue massage following nerve damage has been shown to result in improved blood flow, facial symmetry and smiling.[150,195] Furthermore, previous

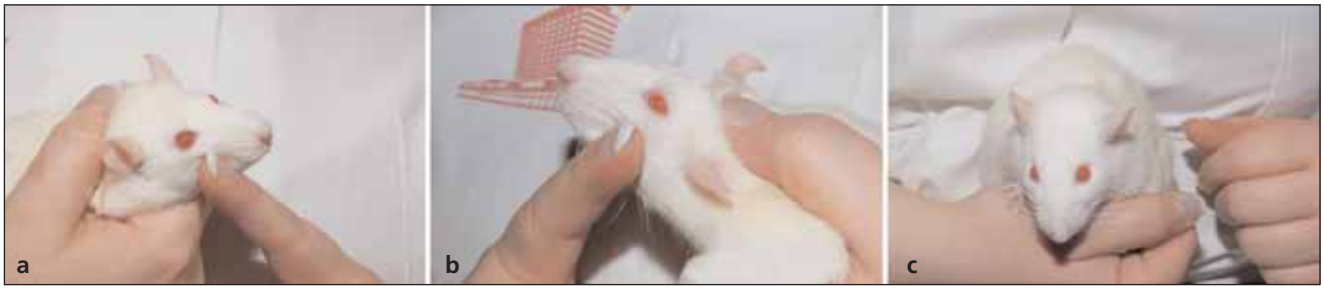


**Figure 8. a:** Manual mechanical stimulation of the right, i.e. ipsilateral to the nerve transection and suture (FFA) vibrissae and whiskerpad muscles. **b:** Manual mechanical stimulation of the left, i.e. contralateral to FFA vibrissae and whiskerpad muscles. **c:** Handling of the animals. Adopted from Angelov et al.[29]

studies in experimental animals have shown that mild electrical stimulation of the denervated soleus muscle inhibits intramuscular sprouting and diminishes motor-end plate polyinnervation.[179,180] Based on these findings, Angelov et al.[29] previously tested the effect of manual stimulation (MS), namely gently stroking the whisker pads by hand for 5 minutes a day for 2 months following FFA in rats. Faint signs of vibrissal motor performance were first noted at 4-5 weeks post FFA and after a further two weeks, recovery was complete with function being indistinguishable from that in intact animals.[29]

Encouraged by the improvement in function by using MS after FFA[29,198] examined whether the same simple rehabilitation technique would be also effective following two other common types of facial nerve reconstruction, hypoglossal-facial anastomosis (HFA) and interpositional nerve grafting (IPNG; **Figure 9).**

The recent experimental evidence for the contribution of manual stimulation to the recovery of vibrissal function following facial nerve injury[29] was confirmed. This next report showed a positive effect after two common types of facial nerve reconstruction, HFA and IPNG. As for FFA, improved recovery of vibrissal motor performance after HFA and IPNG was associated with a significant reduction in the proportion of polyinnervated motor endplates. These results provide new perspectives for implementation of efficient and effective clinical treatment strategies.[198]

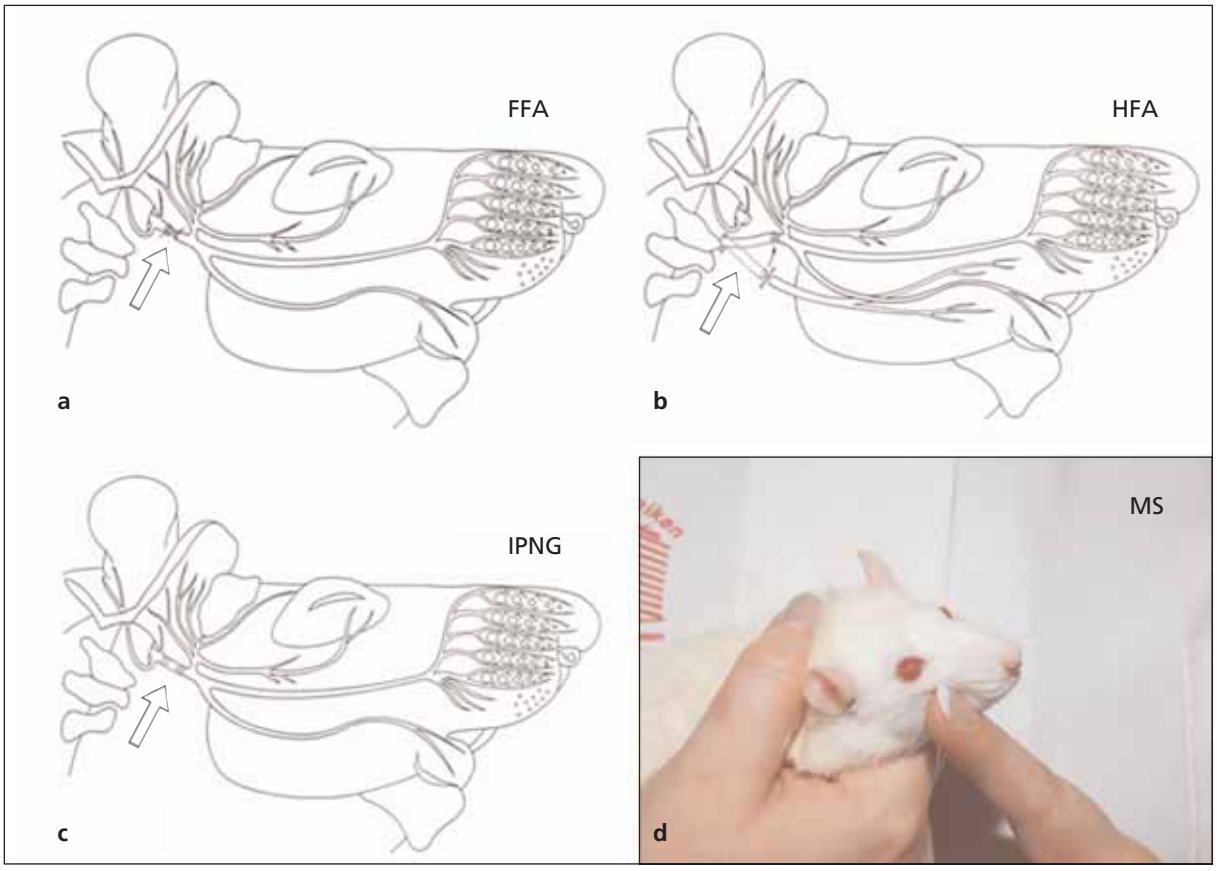


**Figure 9. a-c:** Schematic drawings of the infratemporal portion of the rat facial nerve adapted from Dörfl.[56] **a:** Transection and immediate end-to-end suture of the facial nerve trunk, i.e. facial-facial anastomosis (**FFA**), indicated by an **arrow. b:** Transection of the facial and hypoglossal nerves with subsequent end-to-end suture of the proximal hypoglossal stump to the distal facial fragment (**HFA:** hipoglossofacial anastomosis), indicated by an **arrow. c:** Transection of the facial nerve with subsequent end-to-end suture (**arrow**) with the interpositional nerve graft (**IPNG**) between the proximal and distal facial fragments. **d:** Manual mechanical stimulation (**MS**) of the right, i.e. ipsilateral to the facial nerve transection vibrissae and whiskerpad muscles. Adopted from Guntinas-Lichius et al.[198]

## Manual Stimulation of the Orbicularis Oculi Muscle Improves Eyelid Closure after Facial Nerve Injury

One of the most disturbing deformities in the course of facial palsy is the blinkless eye. In addition to their static expression, these patients suffer many complications from an inability to protect their eyes, including partially obscured visual fields, epiphora (overflow of teardrops upon the cheek), and/or drying of the eye which may in turn lead to keratitis sicca (inflammation of the cornea), corneal abrasions and loss of vision.[199]

Clinical interventions are varied and include surgical approaches whereby static implants passively assist eyelid closure or microsurgical manipulation of nerves and muscles dynamically stimulate active eyelid closure.[200-203] However, results are usually unsatisfactory and functional recovery is often poor.[183,204-206]

Encouraged by the efficacy of manual stimulation in improving function of facial muscles,[29] Bischoff et al.[207] examined whether the same simple rehabilitation technique would also prove effective for another facial muscle, the orbicularis oculi (OOM; **Figure 10).** This muscle is also innervated solely by the facial nerve[53,208,209] and controls eyelid closure and blinking, both of which can be severely compromised by facial nerve injury in humans and with significant consequences.[205,210,211]

Bischoff et al.[207] confirmed recent experimental evidence for the efficacy of manual stimulation following facial nerve injury in promoting recovery of vibrissal function[29,198,212] by showing that manual stimulation also improves motor recovery of another denervated mimic muscle, the orbicularis oculi (OOM). In addition, as for the whisker-pad muscles, improved eyelid closure was associated with a significant reduction in the proportion of poly-innervated motor endplates. Combined, these "proof of principle" findings have immediate implications for clinical rehabilitation following facial nerve injury.

## Manually-Stimulated Recovery of Motor Function after Facial Nerve Injury Requires Intact Sensory Input

As already pointed out, examining the factors limiting restoration of function using the facial nerve in rats as a model, special emphasis was laid on those branches (buccal and mandibular) that supply the whisker pads. Facial nerve cut and anastomosis (FFA) results in robust sprouting at the lesion site and within target muscles.[20-22] A combined quantitative structural and functional approach in the same animals to examine respectively the extent of sprouting and whisker movement led to a number of findings. First, limiting sprouting at the lesion site using antibodies to growth factors did not improve function.[28] Second, facial nerve injury in rats with retinal dystrophy (Royal College of Surgeons strain) resulted in better functional outcome compared to seeing rats, presumably due to forced whisker use.[23] Together, these findings lead to the conclusion that the main site limit-

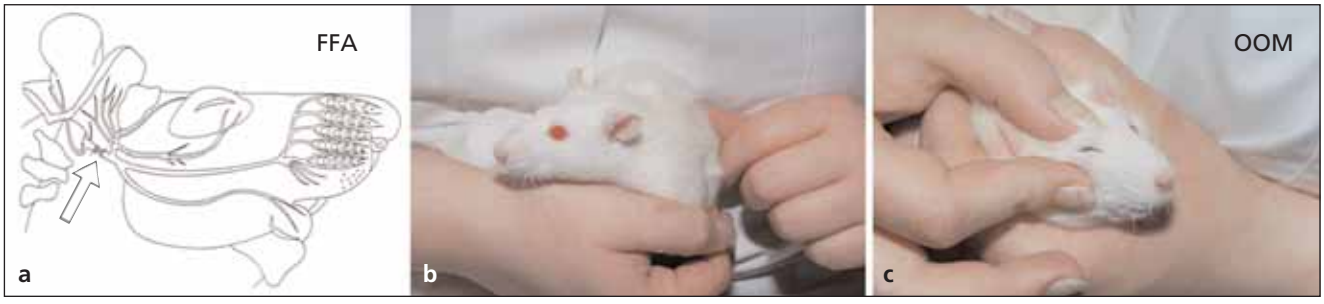


**Figure 10. a:** Schematic drawing of the infratemporal portion of the rat facial nerve. Transection and immediate end-to-end suture of the facial nerve trunk, i.e. facial-facial anastomosis (**FFA**), indicated by an **arrow. b:** Handling of the animals. **c:** Manual mechanical stimulation of the right, i.e. ipsilateral to the facial nerve transection orbicularis oculi muscle (**OOM**). Adopted from Bischoff et al.[207]

ing restoration of whisking was the far periphery where intramuscular sprouting within the whisker pads results in a high proportion of poly-innervated motor end-plates.[28] Indeed, recently it was shown in rats that brief, daily stroking (manual stimulation, MS) of the vibrissal muscles slings following facial-facial anastomosis (FFA), hypoglossal-facial anastomosis (HFA) and inter-positional nerve grafting (IPNG) significantly reduced motor end-plate polyinnervation and concomitantly improved whisking function.[29,198]

Both clinical and experimental data show that recovery of function is better following damage of a purely motor nerve compared to mixed peripheral nerves, that is, those posesssing both motor and sensory axons such as the median nerve.[213-217] Nerve supply to facial muscles has the distinct advantage that the motor and sensory supplies are separate.[218,219] In the case of the facial nerve, sensory feed back occurs via the trigeminal nerve with direct ipsilateral connections between the trigeminal and the facial nucleus in the brainstem.[220-226]

Pavlov et al.[212] took a two step approach to examine the role of afferent inputs. First, they estimated, using synaptophysin immunohistochemistry, the influence of MS on the afferent synaptic input to the facial nucleus. In addition, the influence of the trigeminal sensory input by extirpating one of its branches, the infraorbital nerve (ION) was tested **(Figure 11).** The procedure ablates sensory input from the vibrissal muscle pads to facial motoneurons. Outcome was determined by measuring the degree of polyinnervation of vibrisal motor end-plates and examining recovery of whisking function.

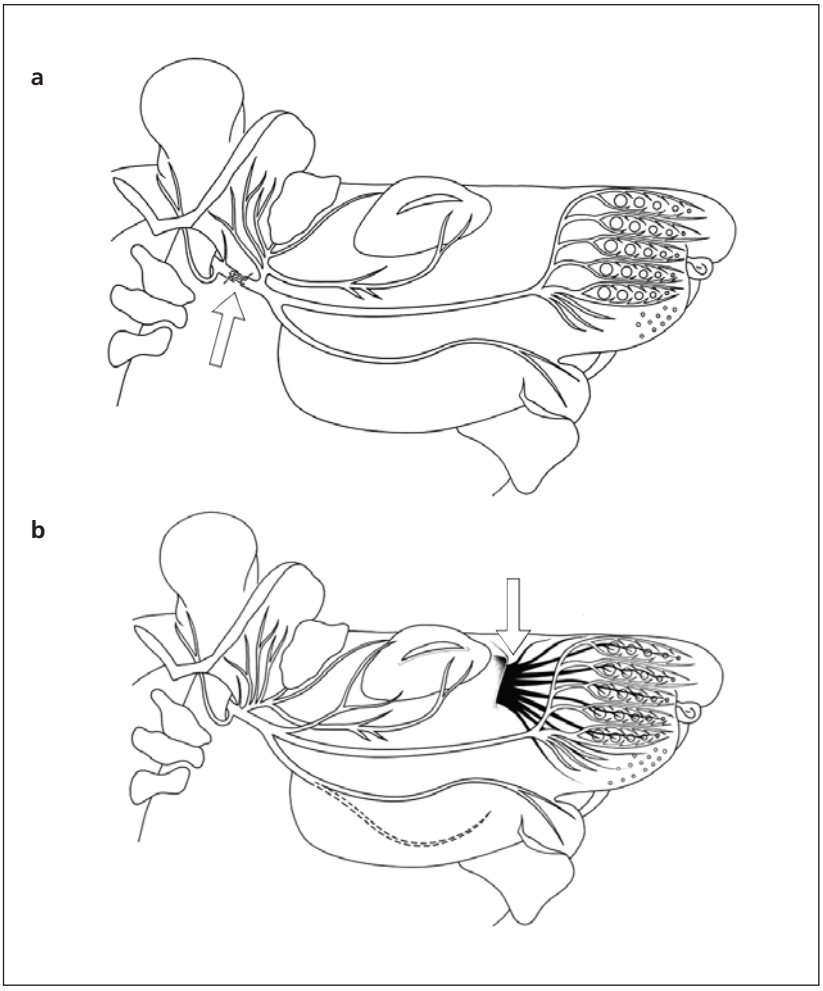


**Figure 11.** Schematic drawings illustrating the infratemporal portion of the facial nerve and the site of its transection and suture (indicated by an **arrow**) in **a** and the close relationship between the peripheral fascicles of the facial nerve and those of the infraorbital nerve (**arrow** indicates the site of excision) in **b.** Adopted from Pavlov et al.[212]

The quantification of the total synaptic input to motoneurons in the facial nucleus (by synaptophysin immunocytochemistry) following FFA with and without subsequent MS showed that, without MS, this input was reduced when compared to intact animals. The number of synaptophysin-positive terminals returned to normal values following MS. Thus, MS appears to counteract the deafferentation of regenerated facial motoneurons.

The elimination of the trigeminal sensory input to facial motoneurons by extirpation of the ipsilateral infraorbital nerve (IONex) showed that, without MS, vibrissal motor performance and pattern of end-plate reinnervation were as aberrant as after FFA without MS. MS did not influence the reinnervation pattern after IONex and functional recovery was even worse than after IONex without MS. Thus, when the sensory system is intact, MS restores normal vibrissal function and reduces the degree of polyinnervation. When afferent inputs are abolished, these effects are eliminated or even reversed. Pavlov et al.[212] concluded that rehabilitation strategies must be carefully designed to take into account the extent of motor and/or sensory damage.

## Recovery of Whisking Function Promoted by Manual Stimulation of the Vibrissal Muscles after Facial Nerve Injury Requires Insulin-like Growth Factor 1 (IGF-1)

A number of factors have been identified which improve the accuracy of reinervation. Stimulating muscles with flaccid paralysis by a variety of means (e.g. electrical stimulation, mechanical stimulation, exercise) inhibits intramuscular sprouting and diminishes motor-end-plate polyinnervation.[179,227] Similarly, it has been recently shown that manual stimulation (MS) of denervated whisker pads after facial nerve injury reduces the amount of terminal sprouting; more accurate reinnervation patterns are associated with improved whisking function and blink reflexes.[29,207] Another factor involves terminal Schwann cells (TSC) which, after injury, extend numerous processes that form bridges within target muscles and act as a substrate for terminal sprouts to reach multiple adjacent (rather than single) motor endplates.[228-230] Moreover, both running exercise and electrical stimulation limit the formation of such bridges and therefore improve the accuracy of reinnervation.[47,180]

A number of molecular factors have been identified which underpin the above structural correlates with insulin-like growth factor-1 (IGF-1) being pivotal. It induces muscle regeneration[231-236] and prevents muscle atrophy.[237-240] It is also involved in Schwann cell viability and myelination.[241,242] In addition, it is closely correlated with neuronal responses to injury being up-regulated at the time of axonal sprouting and elongation.[21,243-246] However, addition of exogenous IGF-1 fails to increase the accuracy of regeneration or functional outcomes such as muscle power, motor evoked potentials and conduction velocity.[247,248]

Although IGF-1 mediates exercise-induced anabolic changes in muscle tissue,[249-251] it is not known whether this neurotrophic factor is involved in stimulation-induced changes within regenerating peripheral nerve axons. Recently Kiryakova et al.[252] first examined IFG-1 expression after facial nerve injury and then used the manual stimulation (MS) protocol in IGF-1 deficient mice to examine whether MS could improve accuracy of reinnervation and recovery of function when IGF-1 was diminished.

Kiryakova et al.[252] examined the effect of daily MS for 2 months after FFA in IGF-$1^{+/-}$ heterozygous mice; controls were wild-type (WT) littermates including intact animals. They quantified vibrissal motor performance and the percentage of NMJ bridged by S100-positive TSC **(Figure 12).** There were no differences between intact WT and IGF-$1^{+/-}$ mice for vibrissal whisking amplitude (48° and 49°) or the percentage of bridged NMJ (0%).

After FFA and handling alone (i.e. no MS) in WT animals, vibrissal whisking amplitude was reduced (60% lower than intact) and the percentage of bridged NMJ increased (42% more than intact). MS improved both the amplitude of vibrissal whisking (not significantly different from intact) and the percentage of bridged NMJ (12% more than intact).

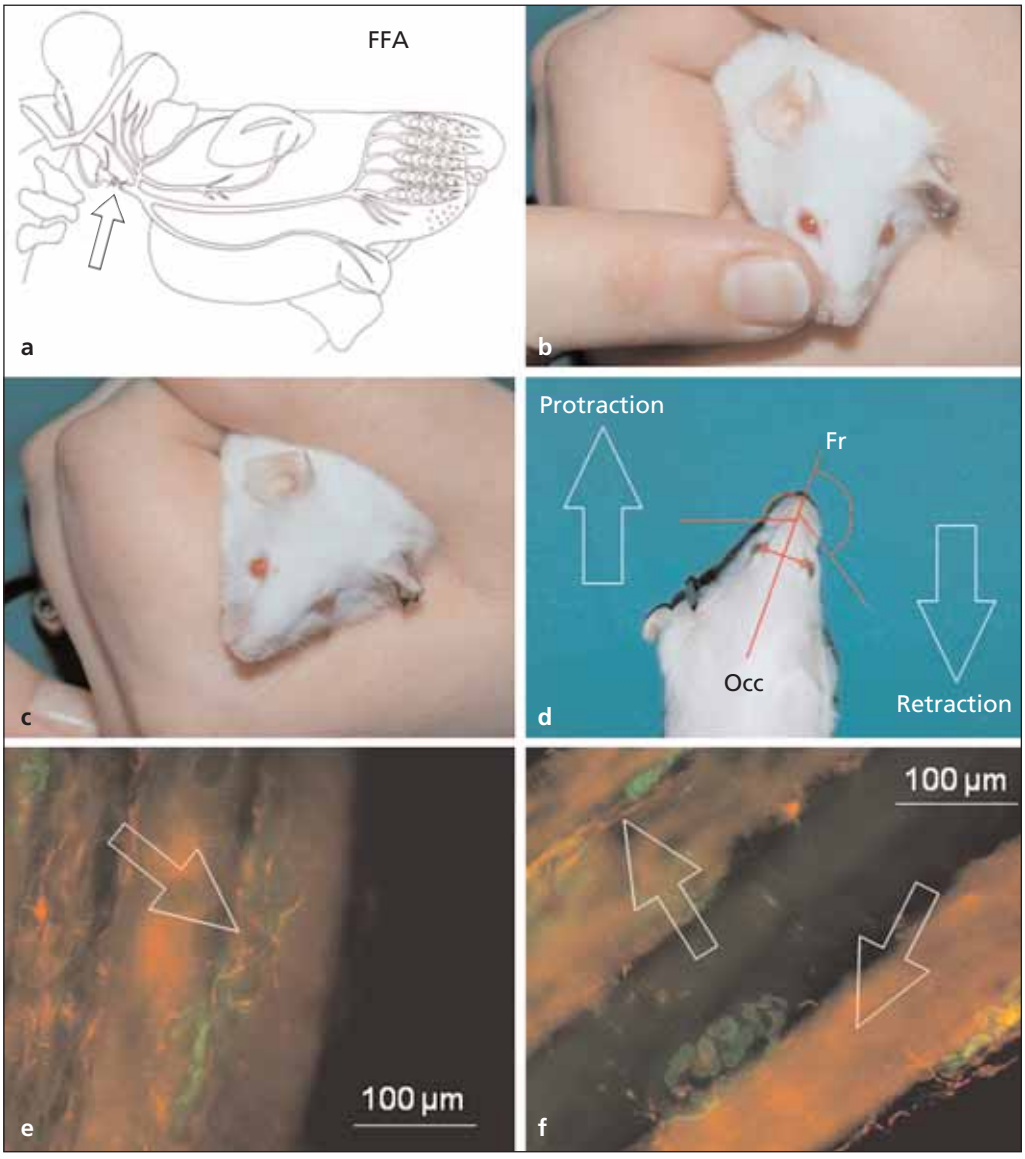


**Figure 12. a:** Schematic drawing of the infratemporal portion of the rat facial nerve. The site of transection and end-to-end suture of the facial nerve trunk, i.e. facial-facial anastomosis (**FFA**) is indicated by an **arrow**. **b:** Manual mechanical stimulation of the right, i.e. ipsilateral to the nerve transection and suture (FFA) vibrissae and whiskerpad muscles. **c:** Handling of the animals. **d:** The developed spatial model allows precise measurement of angles, angular velocity, and angular acceleration on the intact (*left*) and operated side (*right*) during **protraction** and **retraction** of the vibrissae along the sagittal fronto-occipital (**Fr-Occ**) line. **e** and **f:** Typical S-100 positive cytoplasmic bridges (**arrows**) connecting two adjacent motor end-plates in m. levator labii superioris of stimulated WT-mice from group 6 (**e**) and of stimulated IGF-I-deficient mice from group 5 (**f**) 2 months after transection and suture of the facial nerve. Motor end-plates are stained by α-bungarotoxin (Alexa Fluor 488, green fluorescence) and the terminal Schwann cells (TSC) by S-100 protein (Cy3, *red fluorescence*). 30 µm thick cryostat section. Adopted from Kiryakova et al.[252]

After FFA and handling in IGF-1$^{+/-}$ mice, the pattern was similar (whisking amplitude 57% lower than intact; proportion of bridged NMJ 42% more than intact). However, MS did not improve outcome (whisking amplitude 47% lower than intact; proportion of bridged NMJ 40% more than intact). Kiryakova et al.[252] concluded that IGF-I is required to mediate the effects of MS on target muscle reinnervation and recovery of whisking function.

## References


1. Vaughan ED, Richardson D. Facial nerve reconstruction following ablative parotid surgery. *Br J Oral Maxillofac Surg* 1993; 31: 274-80.
2. Ferreira MC, Besteiro JM, Tuma Junior P. Results of reconstruction of the facial nerve. *Microsurgery* 1994; 15: 5-8.
3. Anonsen CK, Trachy RE, Hibbert J, Cummings CW. Assessment of facial reinnervation by use of chronic electromyographic monitoring. *Otolaryngol Head Neck Surg* 1986; 94: 32-6.
4. Goodmurphy GW, Ovalle WK. Morphological study of two human facial muscles: orbicularis oculi and corrugator supercilii. *Clin Anat* 1999; 12: 1-11.
5. Kimura J, Rodnitzky RL, Okawara SH. Electrophysiologic analysis of aberrant regeneration after facial nerve paralysis. *Neurology* 1975; 25: 989-93.
6. Bento RF, Miniti A. Anastomosis of the intratemporal facial nerve using fibrin tissue adhesive. *Ear Nose Throat J* 1993; 72: 663.
7. Baker RS, Stava MW, Nelson KR, May PJ, Huffman MD, Porter JD. Aberrant reinnervation of facial musculature in a subhuman primate: a correlative analysis of eyelid kinematics, muscle synkinesis, and motoneuron localization. *Neurology* 1994; 44: 2165-73.
8. Montserrat L, Benito M. Facial synkinesis and aberrant regeneration of facial nerve. *Adv Neurol* 1988; 49: 211-24.
9. Sumner AJ. Aberrant reinnervation. *Muscle Nerve* 1990; 13: 801-3.
10. Sadjadpour K. Postfacial palsy phenomena: faulty nerve regeneration or ephaptic transmission? *Brain Res* 1975; 95: 403-6.
11. Bratzlavsky M, vander Eecken H. Altered synaptic organization in facial nucleus following facial nerve regeneration: an electrophysiological study in man. *Ann Neurol* 1977; 2: 71-3.
12. Graeber MB, Bise K, Mehraein P. Synaptic stripping in the human facial nucleus. *Acta Neuropathol (Berl)* 1993; 86: 179-81.
13. Moran CJ, Neely JG. Patterns of facial nerve synkinesis. *Laryngoscope* 1996; 106: 1491-6.
14. Aldskogius H, Thomander L. Selective reinnervation of somatotopically appropriate muscles after facial nerve transection and regeneration in the neonatal rat. *Brain Res* 1986; 375: 126-34.
15. Dyck PJ, Hopkins AP. Electron microscopic observations on degeneration and regeneration of unmyelinated nerve fibers. *Brain* 1972; 95: 223-34.
16. Gorio A, Carmignoto G, Finesso M, Polato P Nunzi MG. Muscle reinnervation - II. Sprouting, synapse formation and repression. *Neuroscience* 1983; 8: 403-16.
17. Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 1997; 14: 67-116.
18. Mackinnon SE, Dellon AL, Lundborg G, Hudson AR, Hunter DA. A study of neurotrophism in a primate model. *J Hand Surg Am* 1986; 11: 888-94.
19. Evans PJ, Bain JR, Mackinnon SE, Makino AP, Hunter DA. Selective reinnervation: a comparison of recovery following microsuture and conduit nerve repair. *Brain Res* 1991; 559: 315-21.
20. Dohm S, Streppel M, Guntinas-Lichius O, et al. Local application of extracellular matrix proteins fails to reduce the number of axonal branches after varying reconstructive surgery on the rat facial nerve. *Restor Neurol Neurosci* 2000; 16: 117-26.
21. Streppel M, Azzolin N, Dohm S, et al. Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. *Eur J Neurosci* 2002; 15: 1327-42.
22. Guntinas-Lichius O, Angelov DN, Tomov TL, Dramiga J, Neiss WF, Wewetzer K. Transplantation of olfactory ensheathing cells stimulates the collateral sprouting from axotomized adult rat facial motoneurons. *Exp Neurol* 2001; 172: 70-80.
23. Tomov TL, Guntinas-Lichius O, Grosheva M, et al. An example of neural plasticity evoked by putative behavioral demand and early use of vibrissal hairs after facial nerve transection. *Exp Neurol* 2002; 178: 207-18.
24. Popratiloff AS, Neiss WF, Skouras E, Streppel M, Guntinas-Lichius O, Angelov DN. Evaluation of muscle re-innervation employing pre- and post-axotomy injections of fluorescent retrograde tracers. *Brain Res Bull* 2001; 54: 115-123.
25. Guntinas-Lichius O, Wewetzer K, Tomov TL, et al. Transplantation of olfactory mucosa minimizes axonal branching and promotes the recovery of vibrissae motor performance after facial nerve repair in rats. *J Neurosci* 2002; 22: 7121-31.
26. Skouras E, Popratiloff AS, Guntinas-Lichius O, et al. Altered sensory input improves the accuracy of muscle reinnervation. *Restor Neurol Neurosci* 2002; 20: 1-14.
27. Angelov DN, Skouras E, Guntinas-Lichius O, et al. Contralateral trigeminal nerve lesion reduces polyneuronal muscle innervation after facial nerve repair in rats. *Eur J Neurosci* 1999; 11: 1369-78.
28. Guntinas-Lichius O, Irintchev A, Streppel M, et al. Factors limiting motor recovery after facial nerve transection in the rat: combined structural and functional analyses. *Eur J Neurosci* 2005; 21: 391-402.
29. Angelov DN, Ceynowa M, Guntinas-Lichius O, et al. Mechanical stimulation of paralyzed vibrissal muscles following facial nerve injury in adult rat promotes full recovery of whisking. *Neurobiol Disease* 2007; 26: 229-42.
30. Büngner O. Ueber die Degenerations- und Regenerationsvorgänge am Nerven nach Verletzungen. *Beitr pathol Anat allg Pathol* 1891; 10: 21-393.
31. Bisby MA. Regeneration of peripheral nervous system axons. In: Waxman SG, Kocsis JD, Stys PK, eds. The Axon. New York: Oxford University Press Inc; 1995. p. 553-78.
32. Esslen E. Electromyographic findings on two types of misdirection of regenerating axons. *Electroencephalogr Clin Neurophysiol* 1960; 12: 738-41.

33. Ito M, Kudo M. Reinnervation by axon collaterals from single facial motoneurons to multiple muscle targets following axotomy in the adult guinea pig. *Acta Anat* 1994; 151: 124-30.
34 . Shawe GD. On the number of branches formed by regenerating nerve fibers. *Br J Surg* 1954; 42: 474-88.
35. Morris J, Hudson AR, Weddell G. Study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. II. The development of the "regenerating unit". *Z Zellforsch mikrosk Anat* 1972; 124: 103-30.
36. Friede RL, Bischhausen R. The fine structure of stumps of transected nerve fibers in subserial sections. *J Neurol Sci* 1980; 44: 181-203.
37. Brown MC, Holland RL, Hopkins WG, Keynes RJ. An assessment of the spread of the signal for terminal sprouting within and between muscles. *Brain Res* 1981; 210: 145-51.
38. Rich MM, Lichtman JW. *In vivo* visualization of pre- and postsynaptic change during synapse elimination in reinnervated mouse muscle. *J Neurosci* 1989; 9: 1781-1805.
39. Angelov DN, Neiss WF, Streppel M, Andermahr J, Mader K, Stennert E. Nimodipine accelerates axonal sprouting after surgical repair of rat facial nerve. *J Neurosci* 1996; 16: 1041-8.
40. Hennig R, Dietrichs E. Transient reinnervation of antagonistic muscles by the same motoneuron. *Exp Neurol* 1994; 130: 331-6.
41. Mackinnon SE, Dellon AL, Obrien JP. Changes in nerve fiber diameters distal to a nerve repair in the rat sciatic nerve model. *Muscle Nerve* 1991; 14: 1116-22.
42. Madison RD, Archibald SJ, Lacin R, Krarup C. Factors contributing to preferential motor reinnervation in the primate peripheral nervous system. *J Neurosci* 1999; 19: 11007-16.
43. Grimby G, Einarsson G, Hedberg M, Aniansson A. Muscle adaptive changes in post-polio subjects. *Scand J Rehabil Med* 1989; 21: 19-26.
44. Trojan DA, Gendron D, Cashman NR. Electrophysiology and electrodiagnosis of the post-polio motor unit. *Orthopedics* 1991; 14: 1353-61.
45. Son YJ, Trachtenberg JT, Thompson WJ. Schwann cells induce and guide sprouting and reinnervation of neuromuscular junctions. *Trends Neurosci* 1996; 19: 280-5.
46. English AW. Cytokines, growth factors and sprouting at the neuromuscular junction. *J Neurocytol* 2003; 32: 943-60.
47. Tam SL, Gordon T. Mechanisms controlling axonal sprouting at the neuromuscular junction. *J Neurocytol* 2003; 32: 961-74.
48. Gordon T, Hegedus J, Tam SL. Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. *Neurol Res* 2004; 26: 174-85.
49. Hayworth CR, Moody SE., Ghodosh LA, Krieg P, Rimer M, Zhompson WJ. Induction of neuregulin signaling in mouse Schwann cells *in vivo* mimics responses to denervation. *J Neurosci* 2006 26: 6873-84.
50. Brushart TM. Motor axons preferentially reinnervate motor pathways. *J Neurosci* 1993; 13: 2730-8.
51. Papez JW. Subdivisions of the facial nucleus. *J Comp Neurol* 1927; 43: 159-91.
52. Martin MR, Lodge D. Morphology of the facial nucleus of the rat. *Brain Res* 1977; 123: 1-12.
53. Semba K, Egger MD. The facial "motor" nerve of the rat: control of vibrissal movement and examination of motor and sensory components. *J Comp Neurol* 1986; 247: 144-58.
54. Martin MR, Lodge D, Headley PM, Biscoe TJ. Pharmacological studies of facial motoneurones in the rat. *Eur J Pharmacol* 1977; 42: 291-8.
55. Greene EC. Anatomy of the Rat. New York: Hafner Publishing Co. Inc.; 1955. p. 115-7.
56. Dörfl J. The innervation of the mystacian region of the white mouse. A topographical study. *J Anat* 1985; 142: 173-84.
57. Shohara E, Sakai A. Localization of motoneurons innervating deep and superficial facial muscles in the rat: a horseradish peroxidase and electrophysiologic study. *Exp Neurol* 1983; 81: 14-33.
58. Perry VH, Brown MC. Role of macrophages in peripheral nerve degeneration and repair. *Bioessays* 1992; 14: 401-6.
59. Hirata K, Kawabuchi M. Myelin phagocytosis by macrophages and non-macrophages during Wallerian degeneration. *Microsc Res Tech* 2002; 57: 541-7.
60. McPhail LT, Stirling DP, Tetzlaff W, Kwiecien JM, Ramer MS. The contribution of activated phagocytes and myelin degeneration to axonal retraction/dieback following spinal cord injury. *Eur J Neurosci* 2004; 20: 1984-94.
61. Bray GM, Aguayo AJ. Regeneration of peripheral unmyelinated nerves. Fate of the axonal sprouts which develop after surgery. *J Anat* 1974; 117: 517-29.
62. King CE, Canty AJ, Vickers JC. Alterations in neurofilament associated with reactive brain changes and axonal sprouting following acute physical injury to the rat neocortex. *Neuropathol Appl Neurobiol* 2001; 27: 115-26.
63. McHale MK, Hall GF, Cohen MJ. Early cytoskeletal changes following injury of giant spinal axons in the lamprey. *J Comp Neurol* 1995; 353: 25-37.
64. Sjoberg J, Kanje M. The initial period of peripheral nerve regeneration and the importance of the local environment for the conditioning lesion effect. *Brain Res* 1990; 529: 79-84.
65. Borgens RB. Voltage gradients and ionic currents in injured and regenerating axons. *Adv Neurol* 1988; 47:51-66.
66. Ziv NE, Spira ME. Localized and transient elevations of intracellular Ca2+ induce the dedifferentiation of axonal segments into growth cones. *J Neurosci* 1997; 17: 3568-79.
67. Fawcett JW, Keynes RJ. Peripheral nerve regeneration. *Ann Rev Neurosci* 1990; 13: 43-60.

68. Challacombe JF, Snow DM, Letourneau PC. Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue. *J Cell Sci* 1996; 109: 2031-40.
69. Angelov DN, Neiss WF, Gunkel A, Streppel M, Guntinas-Lichius O, Stennert E. Nimodipine-accelerated hypoglossal sprouting prevents the postoperative hyperinnervation of target muscles after hypoglossal-facial anastomosis in the rat. *Restor Neurol Neurosci* 1997; 11: 109-21.
70. Rosen JM, Padilla JA, Nguyen KD, Padilla MA, Sabelman EE, Pham HN. Artificial nerve graft using collagen as an extracellular matrix for nerve repair compared with suture autograft in a rat model. *Ann Plast Surg* 1990; 25: 375-87.
71. Borkenhagen M, Clemence JF, Sigrist H, Aebischer P. Three-dimensional extracellular matrix engineering in the nervous system. *J Biomed Mater Res* 1998; 40: 392-400.
72. Madison RD, da Silva CF, Dikkes P, Chiu TH, Sidman RL. Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and a laminin-containing gel. *Exp Neurol* 1985; 88: 767-72.
73. Madison RD, da Silva CF, Dikkes P, Sidman RL, Chiu TH. Peripheral nerve regeneration with entubulation repair: comparison of biodegradeable nerve guides versus polyethylene tubes and the effects of a laminin-containing gel. *Exp Neurol* 1987; 95: 378-90.
74. Pesheva P, Probstmeier R, Skubitz APN, McCarthy JB, Furcht LT, Schachner M. Tenascin-R (J1 160/180) inhibits fibronectin-mediated adhesion - functional relatedness to tenascin-C. *J Cell Sci* 1994; 107: 2323-33.
75. Taylor J, Pesheva P, Schachner M. Influence of janusin and tenascin on growth cone behaviour *in vitro*. *J Neurosci Res* 1993; 35: 347-62.
76. Hristov T, Guntinas-Lichius O, Smith G, Angelov DN. Focal application of the cell signaling molecules semaphorin-3A and neuropilin-1 fails to improve recovery of function after peripheral nerve lesion. 22. Arbeitstagung der Anatomischen Gesellschaft, Würzburg, 28-30. September 2005. p. 62. [Abstract]
77. Semba K, Szechtman H, Komisaruk BR. Synchrony among rhythmical facial tremor, neocortical "alpha" waves, and thalamic non-sensory neuronal bursts in intact awake rats. *Brain Res* 1980; 195: 281-98.
78. Bermejo R, Harvey M, Gao P, Zeigler HP. Conditioned whisking in the rat. *Somatosens Mot Res* 1996; 13: 225-33.
79. Komisaruk BR. Synchrony between lymbic system theta activity and rhythmical behaviour in rats. *J Comp Physiol Psychol* 1970; 70: 482-92.
80. Carvell GE, Simons DJ. Biometric analysis of vibrissal tactile discrimination in the rat. *J Neurosci* 1990; 10: 2638-48.
81. Berg RW, Kleinfeld D. Rhythmic whisking by rat: retraction as well as protraction of the vibrissae is under active muscular control. *J Neurophysiol* 2003; 89: 104-17.
82. Streppel M, Angelov DN, Guntinas-Lichius O, et al. Slow axonal regrowth but extreme hyperinnervation of target muscle after suture of the facial nerve in aged rats. *Neurobiol Aging* 1998; 19: 83-8.
83. Reynolds ML, Woolf CJ. Terminal Schwann cells elaborate extensive processes following denervation of the motor endplate. *J Neurocytol* 1992; 21: 50-66.
84. Jergovic D, Stal P, Lidman D, Lindvall B, Hildebrand C. Changes in a rat facial muscle after facial nerve injury and repair. *Muscle Nerve* 2001, 24: 1202-12.
85. Ijkema-Paassen J, Meek MF, Gramsbergen A. Reinnervation of muscles after transection of the sciatic nerve in adult rats. *Muscle Nerve* 2002; 25: 891-7.
86. Grant GA, Rostomily RR, Kim DK, et al. Delayed facial palsy after resection of vestibular schwannoma. *J Neurosurg* 2002; 97: 93-6.
87. Choi D, Raisman G. Somatotopic organization of the facial nucleus is disruprted after lesioning and regeneration of the facial nerve: the histological representation of synkinesis. *Neurosurgery* 2002; 50: 355-62.
88. English AW. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *J Comp Neurol* 2005; 490: 427-41.
89. Guan KL, Rao Yi. Signalling mechanisms mediating neuronal responses to guidance cues. *Nat Rev Neurosci* 2003; 4: 941-56.
90. Hahn CM, Kleinholz H, Koester MP, Grieser S, Thelen K, Pollerberg GE. Role of cyclin-dependent kinase 5 and its activator P35 in local axon and growth cone stabilization. *Neuroscience* 2005; 134: 449-65.
91. Peeva GP, Angelova SK, Guntinas-Lichius O, et al. Improved outcome of facial nerve repair in rats is associated with enhanced regenerative response of motoneurons and augmented neocortical plasticity. *Eur J Neurosci* 2006; 24: 2152-62.
92. Hirokawa N, Takemura R. Molecular motors and mechanisms of directional transport in axons. *Nat Rev Neurosci* 2005; 6: 201-14.
93. Spira ME, Oren R, Dormann A, Gitler D. Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured Aplysia neurons. *J Comp Neurol* 2003; 457: 293-312.
94. Kalil K, Dent EW. Touch and go: guidance cues signal to the growth cone cytoskeleton. *Curr Opin Neurobiol* 2005; 15: 521-6.
95. Tetzlaff W, Alexander SW, Miller FD, Bisby MA. Response of facial and rubrospinal neurons to axotomy:changes in mRNA expression for cytoskeletal proteins and GAP-43. *J Neurosci* 1991; 11: 2528-44.
96. Tetzlaff W, Bisby MA, Kreutzberg GW. Changes in cytoskeletal proteins in the rat facial nucleus following axotomy. *J Neurosci* 1988; 8: 3181-9.
97. Tetzlaff W, Graeber MB, Bisby MA, Kreutzberg GW. Increased glial fibrillary acidic protein synthesis in astrocytes during retrograde reaction of the rat facial nucleus. *Glia* 1988; 1: 90-5.

98. Tetzlaff W, Leonard C, Krekoski CA, Parhad IM, Bisby MA. Reductions in motoneuronal neurofilament synthesis by successive axotomies: a possible explanation for the conditioning lesion effect on axon regeneration. *Exp Neurol* 1996; 139: 95-106.

99. Schaefer AW, Kabir N, Forscher P. Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. *J Cell Biol* 2002; 158: 139-52.

100. Fukata Y, Itoh TJ, Kimura T, et al. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nature Cell Biol* 2002; 4: 583-91.

101. Baas PW, Ahmad FJ. The transport properties of axonal microtubules establish their polarity orientation. *J Cell Biol* 1993; 120: 1427-37.

102. Tanaka E, Ho T, Kirschner MW. The role of microtubule dynamics in growth cone motility and axonal growth. *J Cell Biol* 1995; 128: 139-55.

103. Williamson T, Gordon-Weeks PR, Schachner M, Taylor J. Microtubule reorganization is obligatory for growth cone turning. *Proc Natl Acad Sci USA* 1996; 93: 15221-6.

104. Challacombe JF, Snow DM, Letourneau PC. Dynamic microtubule ends are required for growth cone turning to avoid an inhitory guidance cue. *J Neurosci* 1997; 17: 3085-95.

105. Pan YA, Misgeld T, Lichtman JW, Sanes JR. Effects of neurotoxic and neuroprotective agents on peripheral nerve regeneration assayed by time-lapse imaging *in vivo*. *J Neurosci* 2003; 23: 11479-88.

106. Grosheva M, Guntinas-Lichius O, Angelova SK, et al. Local stabilization of microtubule assembly improves recovery of facial nerve function after repair. *Exp Neurol* 2008; 209: 131-44.

107. Bunge RP. Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. *Curr Opin Neurobiol* 1993; 3: 805-9.

108. Wewetzer K, Verdú E, Angelov DN, Navarro X. Olfactory ensheathing glia and Schwann cells: two of a kind? *Cell Tissue Res* 2002; 309: 337-45.

109. Kwon YW, Gurney ME. Systemic injections of ciliary neurotrophic factor induce sprouting by adult motor neurons. *Neuroreport* 1994; 5: 789-92.

110. Gallo G, Letourneau PC. Localized sources of neurotrophins initiate axon collateral sprouting. *J Neurosci* 1998; 18: 5403-14.

111. Davies AM. Neurotrophins: neurotrophic modulation of neurite growth. *Curr Biol* 2000; 10: 198-200.

112. Siegel SG, Patton B, English AW. Ciliary neurotrophic factor is required for motoneuron sprouting. *Exp Neurol* 2000; 166: 205-12.

113. Bartolomei JC, Greer CA. Olfactory ensheathing cells: Bridging the gap in spinal cord injury. *Neurosurgery* 2000; 47: 1057-69.

114. Franklin RJM, Barnett SC. Olfactory ensheathing cells and CNS regeneration: the sweet smell of success? *Neuron* 2000; 28: 15-8.

115. Ramon-Cuéto A, Plant GW, Avila J, Bunge M. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J Neurosci* 1998; 18: 3803-15.

116. Verdu E, Navarro X, Gudino-Cabrera G, et al. Olfactory bulb ensheathing cells enhance peripheral nerve regeneration. *Neuroreport* 1999; 10: 1097-101.

117. Cheng H, Cao YH, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. *Science* 1996; 273: 510-3.

118. Li Y, Raisman G. Schwann cells induce sprouting in motor and sensory axons in the adult rat spinal cord. *J Neurosci* 1994;14: 4050-63.

119. Li Y, Field PM, Raisman G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* 1997; 277: 2000-2.

120. Jahagirdar BN, Verfaillie CM. Multipotent adult progenitor cells and stem cell plasticity. *Stem Cell Rev* 2005; 1: 53-9.

121. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143-7.

122. Arnhold S, Klein H, Klinz FJ, et al. Human bone marrow stroma cells display certain neural characteristics and integrate in the subventricular compartment after injection into the liquor system. *Eur J Cell Biol* 2006; 85: 551-65.

123. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats – similarities to astrocyte grafts. *Proc Natl Acad Sci U S A* 1998; 95: 3908-13.

124. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A* 1999; 96: 10711-6.

125. Gage FH. Cell therapy. *Nature* 1998; 392: 18-24.

126. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, et al. Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp Neurol* 2000; 164: 247-56.

127. Dezawa M. Central and peripheral nerve regeneration by transplantation of Schwann cells and transdifferentiated bone marrow stromal cells. *Anat Sci Int* 2002; 77:12-5.

128. Tohill M, Mantovani C, Wiberg M, Terenghi G. Rat bone marrow mesenchymal stem cells express glial markers and stimulate nerve regeneration. *Neurosci Lett* 2004; 362: 200-3.

129. Zhang P, He X, Liu K, et al. Bone marrow stromal cells differentiated into functional Schwann cells in injured rats sciatic nerve. *Artif Cells Blood Substit Immobil Biotechnol* 2004; 32: 509-18.

130. Zhao FQ, Zhang PX, He XJ, et al. Study on the adoption of Schwann cell phenotype by bone marrow stromal cells *in vitro* and *in vivo*. *Biomed Environ Sci* 2005; 18: 326-33.

131. Caddick J, Kingham PJ, Gardiner NJ, Wiberg M, Terenghi G. Phenotypic and functional characteristics of mesenchymal stem cells differentiated along a Schwann cell lineage. *Glia* 2006; 54: 840-9.

132. Chen X, Wang XD, Chen G, Lin WW, Yao J, Gu XS. Study of *in vivo* differentiation of rat bone marrow stromal cells into Schwann cell-like cells. *Microsurgery* 2006; 26: 111-5.

133. Keilhoff G, Goihl A, Langnase K, Fansa H, Wolf G. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. *Eur J Cell Biol* 2006; 85: 11-24.

134. Keilhoff G, Stang F, Goihl A, Wolf G, Fansa H. Transdifferentiated mesenchymal stem cells as alternative therapy in supporting nerve regeneration and myelination. *Cell Mol Neurobiol* 2006; 26: 1235-52.

135. Keilhoff G, Goihl A, Stang F, Wolf G, Fansa H. Peripheral nerve tissue engineering: autologous Schwann cells vs. transdifferentiated mesenchymal stem cells. *Tissue Eng* 2006; 12:1451-65.

136. Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. *Exp Neurol* 2006; 198: 54-64.

137. Spiegel I, Adamsky K, Eshed Y, et al. A central role for Necl4 (SynCAM4) in Schwann cell-axon interaction and myelination. *Nat Neurosci* 2007;10: 861-9.

138. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of *in vitro* differentiated bone-marrow stromal cells. *Eur J Neurosci* 2001; 14: 1771-6.

139. Cuevas P, Carceller F, Dujovny M, et al. Peripheral nerve regeneration by bone marrow stromal cells. *Neurol Res* 2002; 24; 634-8.

140. Cuevas P, Carceller F, Garcia-Gomez I, Yan M, Dujovny M. Bone marrow stromal cell implantation for peripheral nerve repair. *Neurol Res* 2002; 26: 230-2.

141. Mimura T, Dezawa M, Kanno H, Sawada H, Yamamoto I. Peripheral nerve regeneration by transplantation of bone marrow stromal cells-derived Schwann cells in adult rats. *J Neurosurg* 2004; 101: 806-12.

142. Chen CJ, Ou YC, Liao SL, et al. Transplantation of bone marrow stromal cells for peripheral nerve repair. *Exp Neurol* 2007; 204: 443-53.

143. Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. *Glia* 2002; 39:229-36.

144. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. *J Neurosci* 2002; 22: 6623-30.

145. Grosheva M, Guntinas-Lichius O, Arnhold S, et al. Bone marrow-derived mesenchymal stem cells transplantation does not improve quality of muscle reinnervation or recovery of motor function after facial nerve transection in rats. *Biol Chem* 2008; 389: 873-88.

146. Haastert K, Grosheva M, Angelova SK, et al. Schwann cells overexpressing FGF-2 alone or combined with manual stimulation do not promote functional recovery after facial nerve injury. *J Biomed Biotechnol* 2009; article ID 408794. doi:10.1155/2009/408794

147. Cronin GW, Steenerson RL. The effectiveness of neuromuscular facial retraining combined with electromyography in facial paralysis rehabilitation. *Otolaryngol Head Neck Surg* 2003; 128: 534-8.

148. Cole J, Zimmerman S, Gerson S. Nonsurgical neuromuscular rehabilitation of facial muscle paresis. In: Rubin LR, ed. The Paralyzed Face. St. Louis: Mosby-Year Book, Inc.; 1991. p. 107-112.

149. Targan RS, Alon G, Kay SL. Effect of long term electrical stimulation on motor recovery and improvement of clinical residuals in patients with unresolved facial nerve palsy. *Otolaryngol Head Neck Surg* 2000; 122: 246-52.

150. Coulson SE. Physiotherapy rehabilitation following facial nerve paresis. In: Beurskens CHG, van Gelder RS, Heymans PG, Manni JJ, Nicolai JPA, eds. The Facial Palsies. Complementary Approaches. Utrecht: Lemma Publishers; 2005. p. 263-74.

151. Diels HJ. Current concepts in non-surgical facial nerve rehabilitation. In: Beurskens CHG, van Gelder RS, Heymans PG, Manni JJ, Nicolai JPA, eds. The Facial Palsies. Complementary Approaches. Utrecht: Lemma Publishers; 2005. p. 275-83.

152. Al-Majed AA, Neumann CM, Brushart TM, Gordon T. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci* 2000; 20: 2602-8.

153. Brushart TM, Jari R, Verge V, Rohde C, Gordon T. Electrical stimulation restores the specificity of sensory axon regeneration. *Exp Neurol* 2005; 194: 221-9.

154. Geremia NM, Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. *Exp Neurol* 2007; 205: 347-59.

155. Gordon T, Brushart TM, Amirjani N, Chan KM. The potential of electrical stimulation to promote functional recovery after peripheral nerve injury - comparisons between rats and humans. *Acta Neurochir Suppl* 2007; 100: 3-11.

156. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. *Eur J Neurosci* 2000; 12: 4381-90.

157. Brushart TM, Hoffman PN, Royall RM, Murinson BB, Witzel C, Gordon T. Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. *J Neurosci* 2002; 22: 6631-8.

158. Al-Majed AA, Tam SL, Gordon T. Electrical stimulation accelerates and enhances expression of regeneration-associated genes in regenerating rat femoral motoneurons. *Cell Mol Neurobiol* 2004; 24: 379-402.

159. English AW, Schwartz G, Meador W, Sabatier MJ, Mulligan A. Electrical stimulation promotes peripheral axon regeneration by enhanced neuronal neurotrophin signaling. *Dev Neurobiol* 2007; 67: 158-72.

160. Eberhardt KA, Irintchev A, Al-Majed AA, et al. BDNF/TrkB signaling regulates HNK-1 carbohydrate expression in regenerating motor nerves and promotes functional recovery after peripheral nerve repair. *Exp Neurol* 2006; 198: 500-10.

161. Vivó M, Puigdemasa A, Casals L, Asensio E, Udina E, Navarro X. Immediate electrical stimulation enhances regeneration and reinnervation and modulates spinal plastic changes after sciatic nerve injury and repair. *Exp Neurol* 2008; 211: 180-93.

162. Ahlborn P, Schachner M, Irintchev A. One hour electrical stimulation accelerates functional recovery after femoral nerve repair. *Exp Neurol* 2007; 208: 137-44.
163. Skouras E, Merkel D, Grosheva M, et al. Manual stimulation, but not acute electrical stimulation prior to reconstructive surgery, improves functional recovery after facial nerve injury in rats. *Restor Neurol Neurosci* 2009; 27: 237-51.
164. Huizing EH, Mechelse K, Staal A. Treatment of Bell's palsy. An analysis of the available studies. *Acta Otolaryngol* 1981; 92: 115-21.
165. Waxman B. Electrotherapy for treatment of facial nerve paralysis (Bell's palsy). In: Anonymus Health Technology Assessment Reports. 3rd ed. Rockville, MD: National Center for Health Services Research; 1984; 3: 27.
166. Gittins J, Martin K, Sheldrick J, Reddy A, Thean L. Electrical stimulation as a therapeutic option to improve eyelid function in chronic facial nerve disorders. *Invest Ophthalmol Vis Sci* 1999; 40: 547-54.
167. Kern H, Salmons S, Mayr W, Rossini K, Carraro U. Recovery of long-term denervated human muscles induced by electrical stimulation. Muscle Nerve 2005; 31: 98-101.
168. Salmons S, Ashley Z, Sutherland H, Russold MF, Li F, Jarvis JC. Functional electrical stimulation of denervated muscles: basic issues. *Artif Organs* 2005; 29: 199-202.
169. Ashley Z, Salmons S, Boncompagni S, et al. Effects of chronic electrical stimulation on long-term denervated muscles of the rabbit hind limb. *J Muscle Res Cell Motil* 2007; 28: 203-17.
170. Ashley Z, Sutherland H, Russold MF, et al. Therapeutic stimulation of denervated muscles: the influence of pattern. *Muscle Nerve* 2008; 38: 875-86.
171. Salmons S, Jarvis J.C. Functional electrical stimulation of denervated muscles: an experimental evaluation. *Artif Organs* 2008; 32: 597-603.
172. Sinis N, Horn F, Genchev B, et al. Electrical stimulation of paralyzed vibrissal muscles reduces endplate reinnervation and does not promote motor recovery after facial nerve repair in rats. *Ann Anat* 2009; 191: 356-70.
173. Farragher D, Kidd GL, Tallis R. Eutrophic electrical stimulation for Bell's palsy. *Clin Rehabil* 1987; 1: 265-71.
174. Williams HB. A clinical pilot study to assess functional return following muscle stimulation after nerve injury and repair in the upper extremity using a completely implantable electrical system. *Microsurgery* 1996; 17: 597-605.
175. Mosforth J, Taverner D. Physiotherapy for Bell's palsy. *Br Med J* 1958; 9: 675-9.
176. Moller AR, Sen CN. Recordings from the facial nucleus in the rat. signs of abnormal facial muscle response. *Exp Brain Res* 1990; 81: 18-24.
177. Kuroki A, Moller AR, Saito S. Recordings from the facial motonucleus in rats with signs of hemifacial spasm. *Neurol Res* 1994; 16: 389-92.
178. Ishikawa M, Namiki J, Takase M, Ohira T, Nakamura A, Toya S. Effect of repetitive stimulation on lateral spread of F-waves in hemifacial spasm. *J Neurol Sci* 1996; 142: 99-106.
179. Brown MC, Holland RL, Ironton R. Nodal and terminal sprouting from motor nerves. *J Physiol* 1980; 306: 493-510.
180. Love FM, Son YJ, Thompson WJ. Activity alters muscle reinnervation and terminal sprouting by reducing the number of Schwann cell pathways that grow to link synaptic sites. *J Neurobiol* 2003; 54: 566-76.
181. Cohan CS, Kater SB. Suppression of neurite elongation and growth cone motility by electrical activity. *Science* 1986; 232: 1638-40.
182. Brown MC, Holland RL. A central role for denervated tissues in causing nerve sprouting. *Nature* 1979; 282: 724-6.
183. Diels HJ. New concepts in nonsurgical facial nerve rehabilitation. In: Myers E, Bluestone C, eds. Advances in Otolaryngology-Head and Neck Surgery. Chicago: Mosby-Year Book Inc.; 1995. p. 289-313.
184. Eccles JC. Investigations on muscle atrophies arising from disuse and tenotomy. *J Physiol* 1944; 103: 253-66.
185. Sunderland S. Capacity of reinnervated muscles to function efficiently after prolonged denervation. *Arch Neurol Psychiat* 1950; 64: 755-71.
186. Bardosi A, Goebel HH, Stennert E. The ultrastructure of normal and denervated human facial muscles. *Plast Reconstruct Surg* 1987; 79: 171-6.
187. Schwarting S, Schröder M, Stennert E, Goebel HH. Morphology of denervated human facial muscles. *ORL J Otorhinolaryngol Relat Spec* 1984; 46: 248-56.
188. Lieber RL. Skeletal muscle structure and function: implications for rehabilitation and sports medicine. Baltimore: Williams and Wilkins; 1992. p. 210-59.
189. Stennert E, Böschen C, Gunkel A, Goebel HH. Effects of electrostimulation therapy: enzyme-histological and myometric changes in the denrvated musculature. *Eur Arch Otorhinolaryngol* 1994: S37-S41.
190. Lömo T, Westgaard RH. Contractile properties of muscle: control by pattern of muscle activity in the rat. *Proc R Soc Lond B Biol Sci* 1974; 187: 99-103.
191. Mokrusch T, Engelhardt A, Eichhorn K-F, Prischenk G, Sack G, Meindorfer B. Effects of long-impulse electrical stimulation on atrophy and fibre type composition of chronically denervated fast rabbit muscle. *J Neurol* 1990; 237: 29-34.
192. Nix WA. Effects of intermittent high frequency electrical stimulation on denervated EDL muscle of rabbit. *Muscle Nerve* 1990; 13: 580-5.
193. McCulloch KL, Nelson CM. Electrical stimulation and electromyographic biofeedback. In: Umphred DA, ed. Neurological Rehabilitation. St. Louis: Mosby Year Book Inc.; 1995. p. 853-6.
194. Hovind H, Nielsen S. Effect of massage on blood flow in skeletal muscle. *Scand J Rehab Med* 1974; 6: 74-7.
195. Beurskens CHG. The functional rehabilitation of facial muscles and facial expression. In: Castro D, ed. Facial Nerve. Proceedings of the Sixth International Symposium on the Facial Nerve. Amsterdam: Kugler Publications; 1990. p. 509-11.

196. Frach JP, Osterbauer PJ, Fuhr AW. Treatment of Bell's palsy by mechanical force, manually assisted chropractic adjusting and high-voltage electrotherapy. *J Manipulative Physiol Ther* 1992; 15: 596-8.

197. Byrne PJ. Importance of facial expression in facial nerve rehabilitation. *Curr Opin Otolaryngol Head Neck Surg* 2004; 12: 332-5.

198. Guntinas-Lichius O, Hundeshagen G, Paling T, et al. Manual stimulation of facial muscles improves functional recovery after hypoglossal-facial anastomosis and interpositional nerve grafting of the facial nerve in adult rats. *Neurobiol Dis* 2007; 28: 101-12.

199. Choi D, Raisman G. Immune rejection of a facial nerve xenograft does not prevent regeneration and the return of function: an experimental study. *Neuroscience* 2003; 121: 501-7.

200. Lavy J, East CA, Bamber A, Andrews PJ. Gold weight implants in the management of lagophtalmus in facial palsy. *Clin Otolaryngol* 2004; 29: 279-83.

201. Terzis JK. Microsurgical reanimation of the orbicularis oculi. In: Beurskens CHG, van Gelder RS, Heymans PG, Manni JJ, Nicolai JPA, eds. The Facial Palsies. Complementary Approaches. Utrecht: Lemma Publishers; 2005. p. 187-94.

202. Botti G. Superior blepharoplasty. In: Vuyk HD, Lohuis PJFM, eds. Facial Plastic and Reconstructive Surgery. London: Edward Arnold Publishers; 2006. p. 57-67.

203. Mourits MP, Vuyk HD. Eyelid reconstruction. In: Vuyk HD, Lohuis PJFM, eds. Facial Plastic and Reconstructive Surgery. London: Edward Arnold Publishers; 2006. p. 437-53.

204. Fisch U, Lanser MJ. Facial nerve grafting. *Otolaryngol Clin North Am* 1991; 24: 691-708.

205. Guntinas-Lichius O. The facial nerve in the presence of a head and neck neoplasm: assessment and outcome after surgical management. *Curr Opin Otolaryngol Head Neck Surg* 2004; 12: 133-41.

206. May M. Surgical rehabilitation of facial palsy. In: May M, ed. New York: The Facial Nerve. Thieme; 1986. p. 695-777.

207. Bischoff A, Grosheva M, Irintchev A, et al. Manual stimulation of the orbicularis oculi muscle improves eyelid closure after facial nerve injury in adult rats. *Muscle Nerve* 2009; 39: 197-205.

208. Klein BG, Rhoades RW, Jacquin MF. Topography of the facial musculature within the facial (VII) motor nucleus of the neonatal rat. *Exp Brain Res* 1990; 81: 649-53.

209. Gong S, Zhou Q, LeDoux MS. Blink-related sensorimotor anatomy in the rat. *Anat Embryol* 2003; 207: 193-208.

210. Thanos PK, Okajima S, Tiangco DA, Terzis JK. Insulin-like growth factor-I promotes nerve regeneration through a nerve graft in an experimental model of facial paralysis. *Restor Neurol Neurosci* 1999; 15: 57-71.

211. Thanos PK, Tiangco DA, Terzis JK. Enhanced reinnervation of the paralyzed orbicularis oculi muscle after insulin-like growth factor (IGF-I) delivery to a nerve graft. *J Reconstr Microsurg* 2001; 17: 357-62.

212. Pavlov S, Grosheva M, Streppel M, et al. Manually stimulated recovery of motor function after facial nerve injury requires intact sensory input. *Exp Neurol* 2008; 211: 292-300.

213. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. A primate model for chronic nerve compression. *J Reconstr Microsurg* 1985; 1: 185-95.

214. Terzis JK, Papakonstantinou KC. The surgical treatment of brachial plexus injuries in adults. *Plast Reconstr Surg* 2000; 106: 1097-122.

215. Bontioti E, Kanje M, Lundborg G, Dahlin LB. End-to-side nerve repair in the upper extremity of rat. *J Peripher Nerv Syst* 2005; 10: 58-68.

216. Sinis N, Schaller HE, Schulte-Eversum C, et al. Nerve regeneration across a 2-cm gap in the rat median nerve using a resorbable nerve conduit filled with Schwann cells. *J Neurosurg* 2005; 103: 1067-76.

217. Kelly EJ, Jacoby C, Terenghi G, Mennen U, Ljungberg C, Wiberg M. End-to-side nerve coaptation: a qualitative and quantitative assessment in the primate. *J Plast Reconstr Aesthet Surg* 2007; 60: 1-12.

218. Moller AR, Jannetta PJ. Blink reflex in patients with hemifacial spasm. Observations during microvascular decompression operations. *J Neurol Sci* 1986; 72: 171-82.

219. Valls-Sole J, Tolosa ES. Blink reflex excitability cycle in hemifacial spasm. *Neurology* 1989; 39: 1061-6.

220. Kimura J, Lyon LW. Orbicularis oculi reflex in the Wallenberg syndrome: alteration of the late reflex by lesions of the spinal tract and nucleus of the trigeminal nerve. *J Neurol Neurosurg Psychiatry* 1972; 35: 228-33.

221. Erzurumlu RS, Killackey HP. Efferent connections of the brainstem trigeminal complex with the facial nucleus of the rat. *J Comp Neurol* 1979; 188: 75-86.

222. Stennert E, Limberg CH. Central connections between fifth, seventh, and twelfth cranial nerves and their clinical significance. In: Graham MD, House WF, eds. Disorders of the Facial Nerve. New York: Raven Press; 1982. p. 57-65.

223. Hinrichsen CF, Watson CD. Brain stem projections to the facial nucleus of the rat. *Brain Behav Evol* 1983; 22: 153-63.

224. Travers JB, Norgren R. Afferent projections to the oral motor nuclei in the rat. *J Comp Neurol* 1983; 220: 280-98.

225. Isokawa-Akesson M, Komisaruk BR. Difference in projections to the lateral and medial facial nucleus: anatomically separate pathways for rhythmical vibrissa movement in rats. *Exp Brain Res* 1987; 65: 385-98.

226. Sharp FR, Gonzalez MF, Morgan CW, Morton MT, Sharp JW. Common fur and mystacial vibrissae parallel sensory pathways: 14 C 2-deoxyglucose and WGA-HRP studies in the rat. *J Comp Neurol* 1988; 270: 446-69.

227. Brown MC, Ironton R. Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. *Nature* 1977; 265: 459-61.

228. Kang H, Tian L, Thompson W. Terminal Schwann cells guide the reinnervation of muscle after nerve injury. *J Neurocytol* 2003; 32: 975-85.

229. Magill CK, Tong A, Kawamura D, Hayashi A, et al. Reinnervation of the tibialis anterior following sciatic nerve crush injury: a confocal microscopic study in transgenic mice. *Exp Neurol* 2007; 207: 64-74.

230. Madison RD, Sofroniew MV, Robinson GA. Schwann cell influence on motor neuron regeneration accuracy. *Neuroscience* 2009; 163: 213-21.

231. Caroni P, Grandes P. Nerve sprouting in innervated adult skeletal muscle induced by exposure to elevated levels of insulin-like growth factors. *J Cell Biol* 1990;110: 1307-17.

232. Glazner GW, Ishii DN. Insulinlike growth factor gene expression in rat muscle during reinnervation. *Muscle Nerve* 1995; 18: 1433-42.

233. Keller HL, Schneider BStP, Eppihimer LA, Cannon JG. Association of IGF-I and IGF-II with myofiber regeneration *in vivo*. *Muscle Nerve* 1998; 22: 347-54.

234. Di Giulio AM, Germani E, Lesma E, Muller E, Gorio A. Glycosaminoglycans co-administration enhance insulin-like growth factor-I neuroprotective and neuroregenerative activity in traumatic and genetic models of motor neuron disease: a review. *Int J Dev Neurosci* 2000; 18: 339-46.

235. Iida K, Itoh E, Kim DS, et al. Muscle mechano growth factor is preferentially induced by growth hormone in growth hormone-deficient lit/lit mice. *J Physiol* 2004; 560: 341-9.

236. Hayashi S, Aso H, Watanabe K, et al. Sequence of IGF-I, IGF-II and HGF expression in regenerating skeletal muscle. *Histochem Cell Biol* 2004; 12: 427-34.

237. Levinovitz A, Jennische E, Oldfors A, Edwall D, Norstedt G. Activation of insulin-like growth factor II expression during skeletal muscle regeneration in the rat: correlation with myotube formation. *Mol Endocrinol* 1992; 6: 1227-34.

238. Messi ML, Delbono O. Target-derived trophic effect on skeletal muscle innervation in senescent mice. *J Neurosci* 2003; 23: 1351-9.

239. Urushiyama T, Akutsu S, Miyazaki J, Fukui T, Diekwisch TG, Yamane A. Change from a hard to soft diet alters the expression of insulin-like growth factors, their receptors, and binding proteins in association with atrophy in adult mouse masseter muscle. *Cell Tiss Res* 2004; 315: 97-105.

240. Okano T, Yoshida K, Nakamura A, et al. Chronic exercise accelerates the degeneration-regeneration cycle and downregulates insulin-like growth factor-1 in muscle of mdx mice. *Muscle Nerve* 2005; 32: 191-9.

241. Liang G, Cline GW, Macica CM. IGF-1 stimulates de novo fatty acid biosynthesis by Schwann cells during myelination. *Glia* 2007; 55: 632-41.

242. Chattopadhyay S, Shubayev VI. MMP-9 controls Schwann cell proliferation and phenotypic remodeling via IGF-1 and ErbB receptor-mediated activation of MEK/ERK pathway. *Glia* 2009; 57: 1316-25.

243. Pu SF, Zhuang HX, Marsh DJ, Ishii DN. Time-dependent alteration of insulin-like growth factor gene expression during nerve regeneration in regions of muscle enriched with neuromuscular junctions. *Brain Res Mol Brain Res* 1999; 63: 207-16.

244. Zochodne DW, Cheng C. Neurotrophins and other growth factors in the regenerative milieu of proximal nerve stump tips. *J Anat* 2000; 196: 279-83.

245. Tiangco DA, Papakonstantinou KC, Mullinax KA, Terzis JK. IGF-I and end-to-side nerve repair: a dose-response study. *J Reconstr Microsurg* 2001; 17: 247-56.

246. Aberg ND, Gustafson-Brywe K, Isgaard J. Aspects of growth hormone and insulin-like growth gactor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. *ScientificWorldJournal* 2006; 6: 53-80.

247. Welch JA, Kraus KH, Wells MR, Blunt DG, Weremowitz J. Effect of combined administration of insulin-like growth factor and platelet-derived growth factor on the regeneration of transected and anastomosed sciatic nerve in rats. *Am J Vet Res* 1997; 58: 1033-7.

248. Lutz BS, Ma SF, Chuang DC, Wei FC. Effects of systemically applied IGF-1 on motor nerve recovery after peripheral nerve transection and repair in the rat - a functional study. *Hand Surg* 1999; 4: 131-6.

249. Philippou A, Maridaki M, Halapas A, Koutsilieris M. The role of the insulin-like growth factor 1 (IGF-1) in skeletal muscle physiology. *In Vivo* 2007; 21: 45-54.

250. Velloso CP. Regulation of muscle mass by growth hormone and IGF-I. *Br J Pharmacol* 2008; 154: 557-68.

251. Vale RG, de Oliveira RD, Pernambuco CS, de Meneses YP, Novaes JD, de Andrade AD. Effects of muscle strength and aerobic training on basal serum levels of IGF-1 and cortisol in elderly women. *Arch Gerontol Geriatr* 2009 49: 343-7.

252. Kiryakova S, Söhnchen J, Grosheva M, et al. Recovery of whisking function promoted by manual stimulation of the vibrissal muscles after facial nerve injury requires insulin-like growth factor 1 (IGF-1). *Exp Neurol* 2010; 222: 226-34.

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