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Activity-Based Strategies in the Rehabilitation of Peripheral Nerve Injuries

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Additional information is available at the end of the chapter

1. Introduction

Peripheral nerve injuries are an important cause of permanent disability and have a strong negative impact on patients' quality of life. The neurological sequels of peripheral nerve injuries impair daily living and work activities and are often associated with serious complications, such as neuropathic pain [1]. The incidence of traumatic peripheral nerve injuries is higher in young male adults as a result of traffic, occupational and sport accidents [2], with the majority of injuries affecting the upper extremity [1], and a minor, though significant percentage of the nerve injuries damaging the sciatic nerve [3]. Although important advances were made in the surgical treatment, functional outcome following peripheral nerve injury is unsatisfying in most patients.

Experimental work with animal models has revealed several neurobiological mechanisms that are crucial for peripheral nerve regeneration and target reinnervation. This research highlights the role played by cellular and molecular mechanisms in signaling neuron injury and activating nerve's regenerative response. Different strategies have been used to enhance nerve regeneration, which use natural activities to stimulate the damaged nervous system to regenerate, with emphasis on treadmill exercise carried out in the immediate period following nerve damage and repair. In this Chapter we will review some of the work done recently regarding the utilization of activity-based strategies on axonal regeneration, reinnervation and functional recovery, as well as some of the mechanisms underlying these effects. Clinical evidence of the use of exercise and related treatment modalities in the rehabilitation of peripheral nerve damage will also be addressed. The importance of translating animal research to clinical settings and of developing new approaches for rehabilitation after nerve injury that comply with increasing knowledge about neurobiological mechanisms of peripheral nerve

regeneration and functional recovery, and explore the plasticity of the central nervous system, will be mentioned.

2. Neuron early regenerative response

2.1. Injury signaling

Responses in the nerve at the site of injury begin almost immediately following axonal injury. Axotomy initiates a complex and coordinated set of injury signals that convey to neuron soma information regarding the axon injury [4]. Early signals arrive to the cell body in the form of vigorous electrical spiking activity that is generated at the lesion site and propagates up to the neuron soma. Membrane depolarization bursts are accompanied by the opening of voltage-gated and ligand-regulated Na^+ and Ca^{2+} channels and by large transients of intracellular Ca^{2+} concentration increase, which activate several different Ca^{2+}-dependent kinases and raise cAMP levels. The formation of the growth cone, the initial event required for axonal elongation, relies on Ca^{2+} acting as intracellular second messenger and on signaling through the mitogen-activated protein kinase (MAPK) pathway, as well as on protein kinase A (PKA) activation [5]. In addition, Ca^{2+} entering the tip of the axon triggers Ca^{2+}-dependent proteases and enhances protein turnover and cytoskeletal dynamics, promoting growth cone advancement [6].

Axon injury disrupts retrograde axonal transport and deprives the neuron cell body of target-derived molecules, which are supposed to repress the neuronal intrinsic growth ability when neurons are firmly contacting their target organs. Little is known about these negative injury signals but the transforming growth factor beta (TGF-β)/SMAD2/SMAD3 pathway is a candidate. For instance, in primary sensory neurons, growth is repressed by the constitutively expressed inhibitor 5 of protein phophatase 1, which interacts with type 1 TGF-β receptor and triggers activity in the TGF-β/SMAD pathway, a mechanism that is down-regulated by injury [7].

Positive injury signals also make an important contribution to support the regenerative response of neurons after axotomy. At the site of the axonal lesion, several kinases, cytokines and downstream effectors are activated and transported back to the neuron soma [4]. The transport of phosphorylated MAPK from injured axons to the cell body leads to expression of regeneration-associated genes [8]. Likewise, activation of the mammalian-target of rapamycin (mTOR) pathway also promotes neuron growth in injured peripheral nerves, either by rapidly causing phosphorylation of the ribosomal S6 protein, a downstream effector of the mTOR pathway, or by regulating the expression of growth-associated protein(GAP)-43 [9]. Several neural growth factors and cytokines increase in concentration within peripheral nerves in response to injury. These factors include the glycosylated protein (gp)130 cytokine family members: leukemia inhibitory factor (LIF), interleukin-6 (IL-6) and ciliary neurotrophic factor (CNTF) [4], as well as neurotrophins, such as brain-derived neurotrophic factor (BDNF) and respective receptors [10]. These factors also play a crucial role in neuron survival and axonal regeneration [11].

2.2. Wallerian degeneration

Wallerian degeneration refers to the tightly regulated disruption of the distal axon in response to the injury. Myelin disintegration, detachment and proliferation of Schwann cells, macrophages activation, and recruitment of blood borne immune cells accompany Wallerian degeneration and create the conditions in the distal nerve to support axonal growth [12]. Schwann cells, macrophages, and other phagocytes recruited from the blood circulation and entering the nerve through the opened nerve-blood barrier, remove myelin debris and associated inhibitory signals, allowing regenerating axons to penetrate into the distal nerve [13].

In addition to proliferating and phagocytozing myelin debris, Schwann cells also secrete neurotrophic factors that promote axon growth thorough autocrine/paracrine mechanisms, along with cytokines and chemokines that regulate the inflammatory response [12]. For instance, the secretion of pro-inflammatory cytokines (e.g. TNF-α, IL-1, and IL-6) induces activation and secretion of phospholipases (e.g. phospholipase A2) that further increase the production of cytosolic and extracellular signaling molecules that contribute to clearance of myelin remnants in the distal nerve [13].

3. Major limitations for nerve regeneration

Experimental in vivo research highlights three key causes for poor reinnervation: 1) axonal loss of regenerative response [14], 2) inability of denervated distal nerve to support axonal growth [15], and 3) severe muscle atrophy [16, 17]. Growing axons progressively lose their regenerative ability if disconnected with targets. In the rat, the number of regenerating motoneurons declines to around one-third during approximately the first 4 months following axotomy [14]. In this case, however, reinnervated muscles are able to recover from atrophy and muscle strength is regained, as a result of intramuscular nerve sprouting and motor unit enlargement [14]. Chronic axotomized neurons can be stimulated to regenerate by the immunosuppressant FK506, thus reduced regenerative capacity can be overcome by proper stimuli [18].

Contrary to chronic axotomy, distal nerve stump denervation and severe muscle atrophy impede full recovery. Distal nerves that remain denervated for few months display diminished ability to support axonal growth and muscle reinnervation. As a consequence, muscles are only partially reinnervated as a result of decreased number of regenerating axons (see Figure 1). Only about 10 percent of motoneurons are capable of regenerating across a chronically denervated distal nerve [15]. In addition, similar numbers of motoneurons grow across the nerve pathway and reinnervate the muscles, showing the chronic denervated distal nerve as the main reason for severely impaired axonal regeneration and muscle reinnervation. This is likely the result of a decline in the number of Schwann cells, together with diminished ability of these cells to stimulate axon elongation [19]. In fact, Schwann cells reactivation by TGF-β elevates their capacity to support axon regeneration [20].
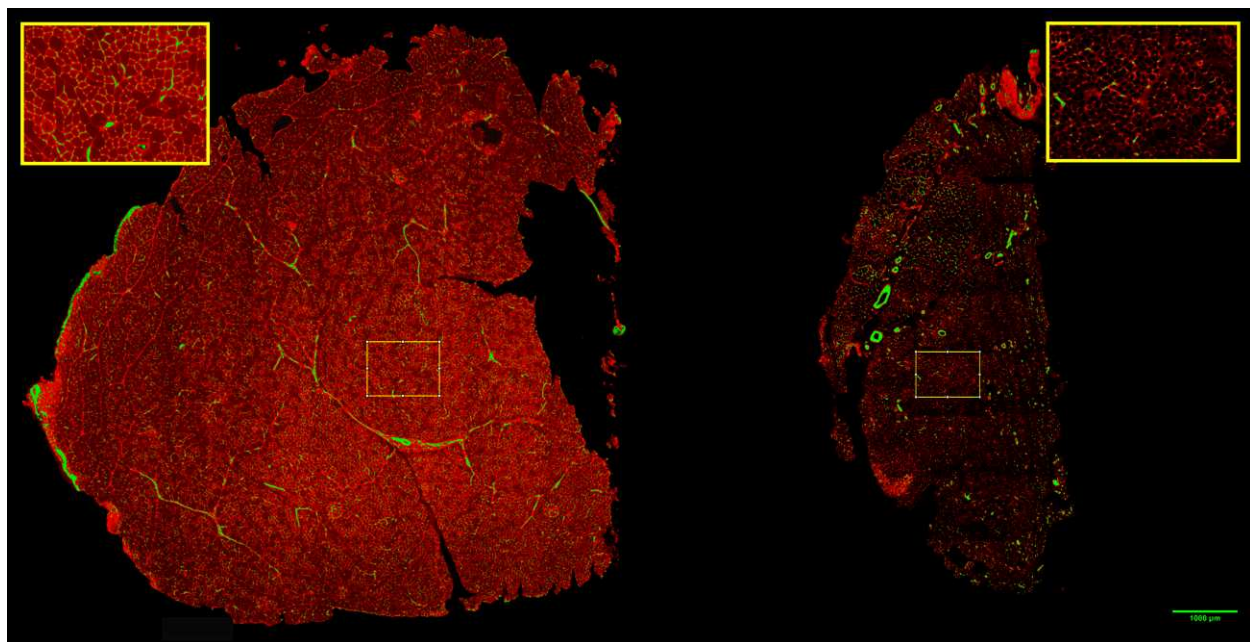
Figure 1. Montages of photomicrographs of transverse cryosections of the tibial anterior muscle from a control rat (left image) and from an animal 20 weeks following sciatic nerve transection and repair with a 10 mm-long nerve autograft (right image). Sections were immunostained for CD31 and fluorescein labeled Griffonia simplicifolia for visualization of blood vessels. Note the severe atrophy of the poorly reinnervated muscle and the extensive loss of capillaries. Scale bar: 1 mm.

Prolonged denervation leads to severe muscle atrophy, muscle fiber necrosis, fibrosis, and endplates disorganization [21, 22]. Extended denervated muscles when reinnervated by fresh axotomized nerves fail in restoring their tetanic force and muscle weight. The size of regenerated muscle units as well as muscle fibers' cross sectional area remain smaller when muscles stay denervated for extended periods of time [16]. Prolonged denervation initially causes atrophy of muscle fibers, but latter there is necrotic muscle changes that lessen the number of muscle fibers [21]. With time, denervated muscles also show impaired myogenesis, which further limits the ability to restore muscle mass even in the case muscles become reinnervated [23, 24] (Figure 2).

Delayed initiation of axonal growth plus misdirection of regenerating axons are additional reasons for poor functional recovery [25-27]. The injury site acts as a barrier that inhibits axonal regeneration and widens the interval of time that different neurons take to successfully initiate their growth (i.e. staggered regeneration). In the mean time, the distal nerve loses part of its ability to support axonal regeneration. Nerve injuries that preserve nerves' connective scaffold show better functional outcome [26]. In crush injuries the endoneurium remains intact along the entire distal nerve stump providing guidance for growing axons to reach their specific targets. Erratic guidance of regenerating axons increases if nerve injury disrupts the endoneurium. In this case, sprouts from regenerating axons may penetrate several different endoneurial pathways and terminate in targets that they formerly did not supply, as in the case of axons that regenerate within nerves leading to a totally different organ than the original

(e.g. skin instead of muscle), or branches of a single motor axon ending up reinnervating muscles with antagonistic function.
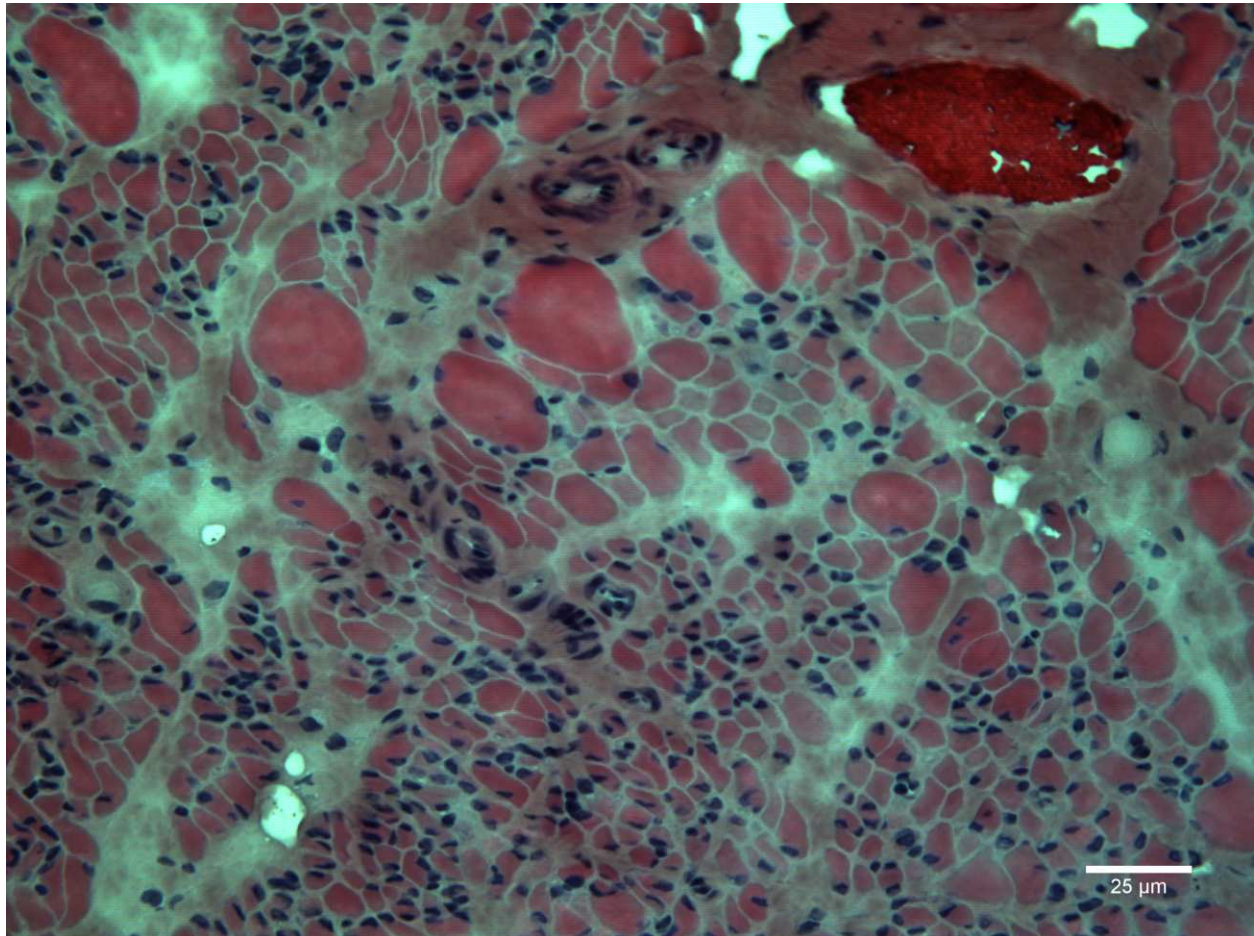
Figure 2. Photomicrograph of hematoxylin-eosin stained transverse cryosection of a poorly reinnervated tibial anterior muscle 20 weeks following sciatic nerve transection and repair with 10 mm-long nerve autograft. Visible a large number of highly atrophied muscle fibers with increased density of myonuclei, intermingled with small clusters of larger muscle fibers. Also visible, the large amount of connective tissue replacing the original muscle tissue. Scale bar: 25 microns.

4. Enhancement of axonal growth

4.1. Brief electrical stimulation

Stimulation of the neuronal activity early following axonal damage might strengthen the intrinsic injury signaling mechanisms and produce a more robust regenerative response. This hypothesis has been tested by using different methods to stimulate the activity of neurons soon following axotomy. Electrical stimulation of cut peripheral nerves applied immediately following axotomy is one of such methods. Brief one-hour, low-frequency electrical stimulation applied by the time of surgery accelerates motoneurons growth in the rat's femoral nerve

and improves reinnervation specificity [28]. Importantly, repeating the electrical stimulation for two weeks postinjury does not result in additional positive effect on axonal regeneration [28]. Tetrodoxin abolishes the effect of electrical stimulation on axonal regeneration, which suggests that action potentials triggering and their propagation to the cell body are required to stimulate axonal regeneration by brief electrical stimulation. Thus, brief electrical stimulation applied immediately following axotomy probably strengthens intrinsic injury signaling, powerfully driving axonal regeneration.

Brief electrical stimulation acutely applied to the cut sciatic nerve also enhances motoneurons regeneration [29]. This effect is most noticed during the first two weeks postinjury, declining to some extent thereafter. However, in the cut and repaired sciatic nerve, electrical stimulation does not improve regeneration precision, therefore a significant proportion of regenerating motoneurons grow along the incorrect pathway, possibly affecting functional outcome negatively [29].

4.2. Treadmill exercise

Treadmill walking/running is the most commonly used form of activity-based experimental treatment within the context of peripheral nerve injury. An important question is whether treadmill exercise enhances axonal regeneration. Although initial studies addressing this question arrived to conflicting results (see ref. [30]), more recent evidence demonstrates that treadmill training accelerates axonal regeneration much in the same way that brief electrical stimulation does [31]. One hour of treadmill running conducted during the first two weeks (5 days/week) following sciatic nerve transection and direct repair in mice increases by four-fold the number of motoneurons that successfully regenerate during that period [32]. By the end of the fourth week of recovery, higher number of regenerated motoneurons could still be observed as a result of treadmill running performed two weeks earlier [32]. The enhancing effect of treadmill training on axonal regeneration can be achieved with different running protocols, ranging from continuous, mild-intensity, prolonged running (e.g. 1 hour/day, 10 m/min), to interval training made of bouts of high-intensity running, separated by periods of recovery (e.g. 4 x 2 min of running at 20 m/min and 5 min of recovery) [31, 33]. In addition to diminishing staggered regeneration, the more natural stimulation provided by treadmill exercise, relative to electrical stimulation, also prevents misrouting of the regenerating sciatic nerve motoneurons [32]. This effect might be crucial in terms of functional outcome considering that misrouting of regenerated neurons underlies pathological manifestations, such as dyskinesia [26]. Improved topographic organization of regenerated motoneurons in response to treadmill exercise is not clearly understood, but is likely related with better synchronized motoneuronal regeneration and higher competition for endoneurial pathways within the distal nerve stumps [32].

Resistance training also enhances regeneration of injured nerves [30]. This kind of exercise consists of performing sets of strong muscle contractions in order to increase muscle strength and augment muscle mass. Compared with resistance training and concurrent training (i.e. resistance and endurance training combined), as well as with sedentary controls, endurance training after sciatic nerve crush injury increases myelin sheath thickness of regenerated nerve

fibers [34]. In addition, endurance training increases the percentage area of the regenerated nerve occupied by myelinated nerve fibers [34]. Resistance training also increases the diameter of the myelinated nerve fibers in crushed sciatic nerves, but only in the segment of the nerve proximal to the injury site [34].

Treadmill exercise and electrical stimulation can be combined, leading to a synergistic effect on promoting nerve regeneration and reinnervation [35]. In rats, and following sciatic nerve transection and direct co-optation, treadmill walking exercise (5 m/min) conducted during the initial four weeks of recovery increases the density and the number of myelinated nerve fibers in the tibial nerve [35]. The same effect is achieved by applying brief electrical stimulation at the time of the surgery. Contrariwise, chronic electrical stimulation fails in improving axonal regeneration. Combining treadmill exercise and brief electrical stimulation enhances the effect of the individual treatments. Low-intensity treadmill exercise in conjunction with acute electrical stimulation also produces faster and enhanced muscle reinnervation. A faster recovery of compound muscle action potential (i.e., M-wave) amplitude, as well as improved M-wave latency, are achieved if the acute electrical stimulation, which is delivered in a single one-hour session immediately after the nerve injury, is strengthened by treadmill exercise throughout the next four weeks. Interestingly, the effect of the combined acute electrical stimulation and treadmill exercise treatment is seen mostly in dorsiflexor muscles (e.g., tibialis anterior), which are supplied by the common peroneal branch of the sciatic nerve, compared to plantarflexors (e.g., plantaris muscle) that are innervated by the tibial nerve [35]. In addition, the positive effect of treadmill exercise on muscle reinnervation following sciatic nerve transection and repair is significant only past two months of injury and one month from the end of treadmill exercise [35, 36]. Once again, this suggests that treadmill exercise improves reinnervation, and possibly functional outcome as well, by acting upon early axonal regeneration, such as by diminishing staggered axonal regeneration and by raising the rate of axonal growth.

4.3. Passive mobilization

Passive mobilization is usually employed to maintain joint range of motion in paralyzed limbs as a result of peripheral nerve injury. In cases patients recover from total paralysis, as a result of successful reinnervation, passive mobilization is replaced by assisted mobilization in which the therapist aids patients moving their affected joint in the full range of motion. The aim of these treatments is to maintain joint function during the time damaged nerves regenerate and to increase muscle strength once reinnervation takes place.

However, passive mobilization might stimulate axonal regeneration as well. This has been demonstrated by experimental work showing improved end-plate structure, nerve sprouting, and end-plate reinnervation of extensor digitorum longus muscle as a result of passive mobilization undertaken during the immediate days post nerve injury [37]. Following facial nerve neurotmesis and direct cooptation in the rat, whisking function can be restored by stimulating passively the whisker pad for just few minutes daily [38]. Similar outcome is achievable in experimental injuries of the hypoglossal nerve [39]. Passive manual exercise does not alter the number of regenerated motoneurons or the topographic precision of reinnervation

by the facial motoneurons [38]. However, passive activity of the denervated facial territories diminishes the number of Schwann cell bridges connecting end-plates of neighbor muscle fibers and the extent of muscle fibers poly-innervation [38]. Also, recovery of normal whisking function through passive manual treatment only occurs if trigeminal afferents are intact. In such cases, manual stimulation is associated with higher number of synaptic inputs onto facial motoneurons, suggesting that enhanced sensory stimulation achieved with manual stimulation is able to maintain appropriate levels of activity within the trigeminal-facial neural pathways [40].

The regeneration of sciatic motoneurons and muscle reinnervation of rat's hindleg muscles can also be promoted by passive cycling during the first weeks following sciatic nerve neurotmesis and end-to-end repair [36]. The effect of passive cycling on M-wave amplitude and latency, as well as in the magnitude of the electrically-elicited H-reflex, is comparable in magnitude to that of treadmill exercise [36]. These effects of passive mobilization are very relevant regarding translation to clinical practice, since patients are usually unable to undertake active physical exercise early following peripheral nerve injury.

5. Growth factors

The effect provided by activity-based strategies on axonal survival and regeneration may be linked with increased production and release of neurotrophins, growth factors, and hormones.

5.1. Neurotrophins

Neurotrophins are a family of extracellular signaling peptides that include the nerve growth factor (NGF), BDNF and neurotrophin(NT)-3 and NT-4/5. These neurotrophic factors bind to specific high-affinity tropomyosin-receptor kinase (Trk) receptors and to the low affinity p75 receptor. TrkA is the high affinity receptor for NGF, TrkB is the receptor for BDNF and NT-4/5, and TrkC is the receptor for NT-3 [41]. Trk and p75 receptors trigger different downstream intracellular pathways and different cell responses. Low affinity p75 receptors are usually up-regulated after injury and they seem to hamper axonal regeneration [11].

BDNF plays an important role in mediating the effects of physical exercise on synaptic plasticity in the brain [42]. Likewise, BDNF plays a crucial role in axonal regeneration based on several lines of evidence. Following injury, BDNF and their receptors TrkB and p75 are up-regulated in motoneurons and in denervated distal nerve stump [43, 44], although with diffrerences in response magnitude and timing between the two places. In motoneurons, BDNF mRNA expression is rapidly induced following axotomy, then returning to baseline after a few days. In intact nerves, BDNF is expressed at very low levels but in response to nerve transection the amount of BDNF mRNA increases steeply and with a magnitude that varies between different nerves [10]. In response to nerve injury, TrkB gene expression also increases in facial and sciatic motoneurons. The increase in TrkB mRNA levels begin in the immediate days post injury, reaches a three-fold peak increase by the end of the first week, and remains elevated throughout the next three to four weeks [10]. p75 mRNA also is rapidly up-regulated

in axotomized motoneurons. The time course of p75 mRNA response is similar to that of TrkB mRNA but the magnitude is three to four-fold higher [10]. Despite the fast and robust increase in levels of BDNF and respective receptors, exogenous BDNF not always improves regeneration. At low doses, exogenous BDNF does not produce a clear effect on axonal regeneration of acutely injured and repaired peripheral nerves though it is able to promote regeneration of chronic axotomized neurons [45]. Also, large doses of exogenous BDNF impair axonal regeneration, probably by signaling through p75 receptors [45]. In fact, work on transgenic mice provides evidence that the TrkB receptor is necessary for adequate axonal regeneration, whereas signaling through p75 receptor has the opposite effect [11]. Brief electrical stimulation delivered to the proximal nerve stump immediately postinjury produces a fast and large BDNF and TrkB mRNAs response [46].

In the brain and in the spinal cord, BDNF expression is up-regulated by voluntary physical exercise with a magnitude that is in close relationship with the distance traveled [47]. The level of BDNF mRNA in the cell soma, in axons of spinal motoneurons, and in soleus muscle also increases after only a few bouts of exercise [48]. Although there is no direct evidence that physical exercise could increase BDNF levels in axotomized neurons or in the distal nerve stump following injury, the effect of treadmill exercise in promoting axonal regeneration requires BDNF expression by parental motoneurons [49]. In fact, treadmill training allows axons to grow into allografts harvested from Schwann cells BDNF-/-transgenic mice, which otherwise does not occur. However, in transgenic mice whose motoneurons do not express BDNF, axons fail to regenerate into grafts from Schwann cells BDNF-/-donors even when stimulated by treadmill exercise, thus suggesting that treadmill running up-regulates BDNF expression in regenerating motoneurons and that this is, at least in part, the mechanism by which treadmill running stimulates axonal regeneration [49]. BDNF and TrkB expression is also required for the role of passive manual stimulation on facial muscles'reinnervation. In fact, heterozygous deficient BDNF and TrkB mice, unlike their wild type counterparts, are unable to respond favorably to the manual passive treatment [50].

The role of the other neurotrophins, namely NGF, NT-3, NT-4/5, in promoting axonal regeneration is less well established, but simply based on changes in their expression following nerve injury they likely play a less important role compared to BDNF. Following axotomy, expression of NT-3 and NT-4/5, as well as that of TrkC, is rapidly down-regulated in parent motoneurons and in the distal nerve stump, whereas NGF levels increase in the distal nerve stump [10]. Despite being down-regulated, NT-3 treatment increases the number of motoneurons that successfully regenerate through nerve gaps and improves muscle reinnervation, specifically in fast contracting muscles [51, 52].

5.2. IGF-1

Insulin-like growth factor-1 (IGF-1) regulates many of skeletal muscle responses to physical exercise [53]. Muscle fibers increase the expression of IGF-1 in response to contractile activity and mechanical loading. Acting in autocrine/paracrine fashion, IGF-1 regulates muscle protein turnover and proliferation, and survival and differentiation of muscle-resident stem cells, namely satellite cells [53]. Serum levels of IGF-1 also increase as a result of different types of

exercise, including resistance and endurance exercise [54], due to release from the muscle, liver and possibly other non-hepatic tissues, stimulated by growth hormone and by the exercise itself.

Numerous studies confirm the role played by IGF-1 on peripheral nervous system regeneration [55, 56]. Besides being released by muscle fibers, IGF-1 is also expressed in motoneurons and Schwann cells. In the nervous system, IGF-1 has a neuroprotective action and promotes the regeneration of peripheral nerves [57]. *In vitro*, IGF-1 promotes neurite outgrowth of cultured motoneurons, whereas *in vivo* it encourages terminal sprouting in reinnervating muscles [58].

Physical exercise protects against distinct types of brain injury. Such role of physical exercise is believed to be due to a higher quantity of IGF-1 entering the brain as a result of its elevated serum levels by virtue of exercise [59]. Moreover, ischemic brain injury is associated with large decreases in IGF-1 levels in the sciatic nerve, spinal cord, and brain cortex, probably resulting from inactivity [60]. Intramuscular administrations of recombinant IGF-1 increases its levels in the nervous system and in muscles and diminishes brain cortical cell apoptosis and motor dysfunction [60].

IGF-1 is also necessary for whisking function recovery following facial nerve transection and direct cooptation [61]. In heterozygous IGF-1-deficient mice, manual stimulation is ineffective in promoting functional recovery following facial nerve damage [61]. Although it is not possible to conclude from studies conducted in IGF-1 deficient mice that manual stimulation raises IGF-1 levels in the muscle or nerve, they demonstrate the supportive role played by IGF-1 in restoring function following peripheral nerve damage.

5.3. Testosterone

Testosterone and other androgens are important for normal muscle function, particularly regulating muscle anabolism [62]. Androgens also regulate axonal regeneration [63]. The effect of androgens in axonal growth varies with the specific nerve, with androgens playing a more important role in the regeneration of motoneurons in the facial nerve, relative to the sciatic nerve [63]. Androgens act on axonal regeneration through mechanism associated with the androgen receptor, as well as by modulation of stress cells' response, particularly the inhibition of heat shock proteins [63]. In addition, treatment with testosterone propionate leads to increased expression of BDNF and TrkB receptor by regenerating facial motoneurons [64].

The positive effect of treadmill running on axonal regeneration seems to be regulated also by sexual steroids. In mice, the effect of treadmill running on axonal regeneration varies according to gender and treadmill running protocol [65]. In males, but not in females or castrated males, continuous one-hour running each day for two weeks following common fibular nerve cut and repair significantly elevates testosterone serum levels, as well as accelerates axonal elongation [65]. Nonetheless, interval training running, comprised by bouts of intense running interspersed by periods of recovery, promotes axonal regeneration in female mice, although this training leaves serum testosterone baseline levels unchanged in both genders [65]. The use of an aromatase inhibitor, thus blocking the

conversion of testosterone or of its precursors into estradiol, also improves axonal regeneration of common fibular motoneurons in female mice [65], thus suggesting that the failure of continuous exercise in stimulating axonal regeneration in females might be linked to the conversion of testosterone to estradiol or, in alternative, to a direct inhibitory effect of the latter on axonal regeneration.

Notwithstanding the subtleties of the effect of androgens on peripheral nerve regeneration, serum testosterone also increases acutely in response to physical exercise in human subjects [66], and this might aid nerve function.

6. Functional recovery

Although the role of physical exercise in enhancing axonal regeneration seems well established, its effect on functional recovery is less clear. In experimental models of peripheral nerve injury, several different tests are usually employed to evaluate functional recovery, including neurophysiological evaluation of motor reinnervation, muscle force testing, and behavioral tests [67]. In the case of sciatic nerve injury, behavioral test based on footprints, such as the sciatic functional index are commonly used [68]. These tests are non-invasive, relatively simple to perform and suitable for testing at several different and sequential time points.

Although behavioral tests offer relevant data about the recovery process, they gather only limited information regarding movement patterns changes and therefore cannot fully assess functional recovery. Thus, methods for evaluating movement production accurately and to assess performance of complex tasks requiring sensorimotor integration, such as gait, are necessary in peripheral nerve injury research [69].

6.1. Gait analysis

In the rat model, the study of limb kinematics during gait is a powerful means to evaluate functional recovery following peripheral nerve injury. Data of segmental and inter-joint coordination patterns can be combined with recordings of the electromyographical (EMG) activity of muscles [70] and ground reaction forces data [71, 72], providing detailed analysis of movements, including knowledge of joint powers and of the role played by muscles and other forces in producing the recorded movements.

We have used gait analysis in several occasions to assess the effect of different interventions following sciatic nerve injury in the rat, including the use of different tubulization procedures [73], application of biomaterials [74], and use of cellular systems [75, 76].

Figure 3 shows plots of ankle joint kinematics during the gait cycle, including both the stance and swing phases, prior to sciatic nerve transection and repair and at the end of 2 and 20 weeks of recovery in groups of adult male Sprague-Dawley walking across a walkway. Severe changes in ankle kinematics are easily noticed in animals 2 weeks after sciatic nerve transection and repair, which of course are expected due to the paralysis of the muscles crossing this joint. These changes are seen during both the stance and the swing
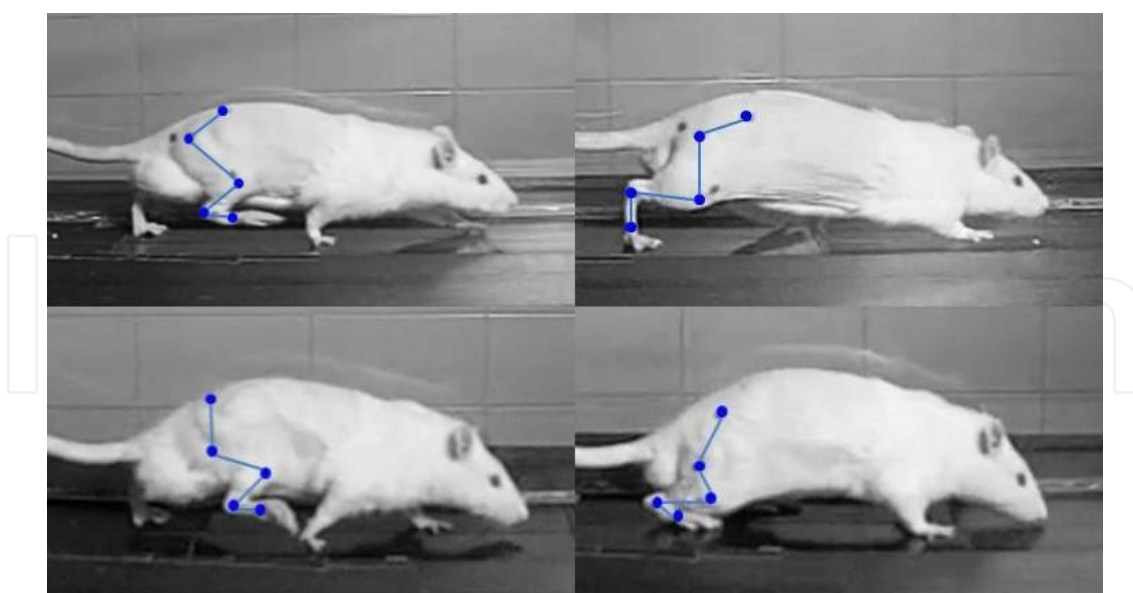
phases of the gait cycle. During the stance phase, and in intact animals, the ankle joint first moves into dorsiflexion, reaching peak dorsiflexion near midstance, next performing plantarflexion until the end of this phase. Two weeks after sciatic nerve injury, ankle peak dorsiflexion angle is greatly increased and there is no plantarflexion during the second half of the stance phase, as a result of the paralysis of the ankle plantarflexor muscles (see also Figure 4). During the swing phase, changes in ankle kinematics are even more pronounced. During this phase of the gait cycle, the paw is lifted from the ground, moved forwardly and then placed again on the ground for the next stepping. Therefore, rats perform a very fast ankle joint action by which they first retract the limb (necessary for paw ground clearance) making ankle dorsiflexion and next extend the limb (to advance and place the paw on the ground again), now doing ankle plantarflexion. This requires fast contractions of the muscles actuating the ankle joint and fine coordination between ankle movement and those of the other limb joints. Acutely sciatic-injured animals are unable to produce such brisk ankle movements during the swing phase and the typical kinematics of the ankle joint is replaced by a short-range and slow plantarflexion that possibly occurs passively. After 20 weeks recovery, ankle kinematics is still deeply altered, despite a slight recovery of ankle plantarflexion near the stance-to-swing transition (Figures 3 and 4). Nevertheless, ankle kinematics during the swing phase remains severely disrupted even by the end of long term recovery (Figures 3 and 4).
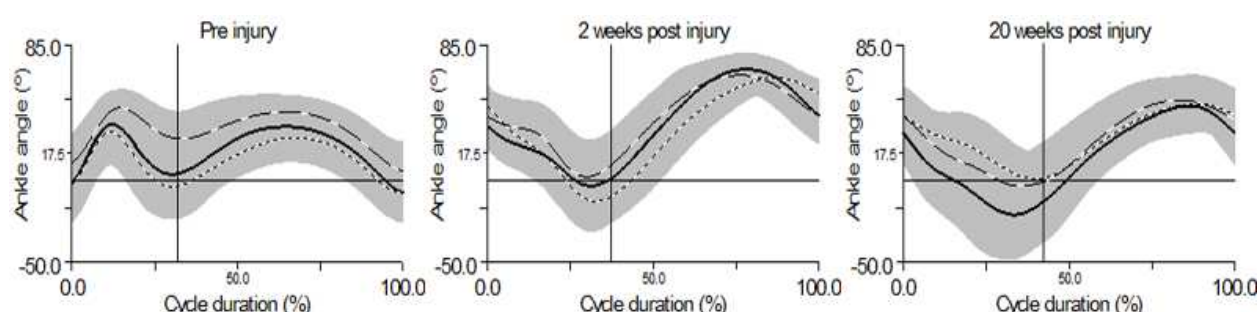
Figure 3. Plots of ankle joint kinematics during the gait cycle prior to sciatic nerve injury and at different times following sciatic nerve transection and repair. Curves represent groups of animals with their transected sciatic nerve treated with end-to-end repair, autograft or tubulization. Shadowed area depicts the standard deviation around the mean curves.

The poor recovery of ankle kinematics during gait following sciatic nerve transection and repair could be explained by limited motoneuron regeneration and lack of muscle reinnervation and strength recovery. However, this does not seem to be the case. In another occasion, we measured the torque produced by the ankle dorsiflexor and plantarflexor muscles in rats 16 weeks following sciatic nerve transection and repair and uninjured animals (unpublished observations; Figure 5). At the end of 16 weeks of recovery from sciatic nerve transection and repair, animals can produce relatively large dorsiflexor and plantarflexor torques. In addition, the torque-angle relationship for both reinnervated dorsiflexor and plantarflexor muscle groups remains largely unchanged.

Figure 4. Photographs of an uninjured rat and a rat 16 weeks after sciatic nerve transection and end-to-end repair collected at approximate instants during the swing (images on the left side) and the stance (images on the right side) phases of the rat walking. Diagrams connecting the approximate centers of rotation of hip, knee, and ankle, as well as the fifth metatarsal head are superimposed. The diagrams are for illustration purposes only and are not supposed to accurately represent the joint angles. Impaired limb kinematics is visible in the sciatic-injured animal during both phases of the gait cycle.

The very limited recovery of normal gait pattern in rats following sciatic nerve transection despite significant recovery in muscle strength, demonstrates that axonal regeneration and muscle reinnervation, although necessary, are not sufficient for functional recovery. The concept of functional recovery is not always straightforward, particularly in the case of nervous system disorders. Following peripheral nerve injury, movement compensations emerge to respond to the disability and to maintain function, as for instance increased knee extension during walking to compensate the abnormal plantigrade gait [77] (Figure 4). Thereby, behavioral compensations are important for functional recovery, since they substitute some lost function, but they also mask disability and may be confounders when evaluating recovery after peripheral nerve injury, particularly when using more rudimentary tests [72]. Furthermore, the repeated use of movement compensations may turn them behaviorally fixed, thus eventually becoming an additional factor hampering further movement normalization [77].

6.2. Promoting spinal cord plasticity by activity-dependent strategies

Peripheral nerve injury causes permanent loss of muscle reflexes and triggers adaptive changes in the spinal cord and probably also in supraspinal sensorimotor centers that disrupt planning and ongoing regulation of movements [78]. Experimental studies with self-reinnervated single muscles illustrate well the role of changed muscle afferent feedback in regulating interjoint coordination and complex locomotor function [79]. Muscle self-reinnervation minimizes axonal misrouting and allows muscle reinnervation and muscle strength recovery. Likewise, hndlimb joint kinematics and patterns of muscle activity recover to normality despite the self-reinnerva-
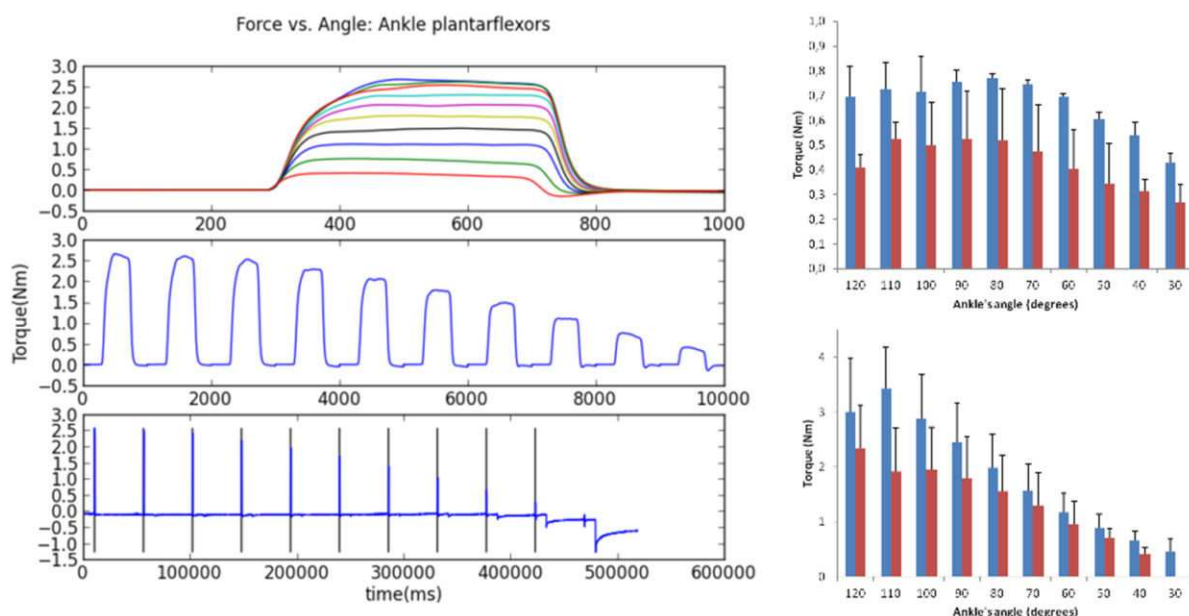
Figure 5. Left panel. Example of recordings of the torque produced during isometric tetanic contractions by the ankle plantarflexor muscles at different joint angles by an anesthetized rat 16 weeks after sciatic nerve transection and end-to-end repair. Ankle plantarflexor torque diminishes progressively with increased plantarflexion angle. In the lower graph, baseline down shifting close to the end of the recording indicates the passive torque generated by the soft tissue around the ankle joint near the end of the plantarflexion range of motion. Right panel. Mean values for ankle dorsiflexor (upper graph) and plantarflexor (lower graph) torque from 5 rats, 16 weeks following sciatic nerve transection and direct end-to-end repair and from equal number of uninjured control animals.

tion of ankle plantarflexors, but altered interjoint coordination appears if biomechanical constraints are imposed, such as walking up or down an incline or at a higher speed [79].

Proprioceptive deficits are the main explanation for changes in limb coordination after self-reinnervarion of ankle joint plantarflexors [78], and likely also in our sciatic nerve-injured animals. Reinnervated muscles are unresponsive to a stretch stimulus by virtue of unsuccessful reinnervation of muscle sensory organs by their specific sensory afferents, inability to rearrange central connections of sensory afferents to match changed target (e.g. Ib afferents changing their target from the Golgi end organ to muscle spindle receptors), and loss of monosynaptic sensory inputs onto motoneurons (i.e., synaptic stripping) [80, 81].

Spinal cord function, and in particular the recovery of central connections mediating muscle reflexes, may be ameliorated by up-conditioning of the H-reflex [82]. The strengthening of the H-reflex response using operant conditioning accelerates the recovery of the M-wave and H-reflex response in the soleus muscle in rats after sciatic nerve transection and repair [82]. Together with restoration of the electrical component of the muscle stretch reflex, H-reflex up-conditioning is associated anatomically with higher number of synaptic terminals established between primary sensory axons and motoneurons in the ventral horn [82].

These results are promising as they show the ability of activity-based interventions to shape spinal cord plasticity and revert, at least to some extent, nonadaptive secondary changes in spinal cord circuitry regulating motor output. A more normal kind of activity, in this case

treadmill exercise also helps in restoring the H-reflex response in rats following sciatic nerve transection and repair, while also contributing to a pattern of muscle activation between antagonistic muscles of the ankle joint during gait better resembling that of uninjured animals [83].

7. Translational research and clinical studies

Although the positive effect of increased stimulation of motor and sensory pathways by natural activities, like treadmill exercise, on nerve regeneration, and possibly also on functional recovery, seems proven by the research conducted with animal models of peripheral nerve injury, similar evidence does not exist in the case of human patients. The lack of clear evidence demonstrating the efficacy of exercise therapy on functional outcomes is reported for conditions such as carpal tunnel syndrome [84], ulnar neuropathy [85], and Bell's palsy [86]. Nevertheless, in peripheral neuropathy, resistance exercise might increase muscle strength in affected muscles [87].

There are several physiotherapy modalities in the treatment of Bell's palsy which can be considered activity-based, like exercise therapy, biofeedback and mirror biofeedback, and relaxation. The results of several controlled randomized trials deny that any of such interventions brings a clear benefit to functional outcome [86, 88]. However, from a single preliminary study, there is evidence that active facial exercises are able to improve disability and diminish the prevalence of synkinesis in chronic facial palsy patients [86].

In carpal tunnel syndrome, exercise approaches are usually centered on nerve gliding and soft tissue mobilization. Other more holistic approaches, such as yoga, have also been attempted. In general, nerve gliding and stretching are considered effective in relieving symptoms of carpal tunnel syndrome by improving blood flow in the nerve, decreasing edema and pressure on the nerve, and mobilize the adherent medial nerve within the carpal tunnel [84]. Some studies report small size effect of exercise on measures of functional outcome in carpal tunnel syndrome, but taking into account studies' quality and the risk of bias, the overall evidence does not support a clear additional benefit of exercise or mobilization interventions on functional outcome in this condition [84]. Notwithstanding, carpal tunnel syndrome is a chronic condition, with periods of symptoms remission alternating with periods of symptoms exacerbation, therefore, with distinct physiopathology from that of peripheral nerve acute injury. However, preliminary results indicate that brief low-frequency electrical stimulation of the median nerve, applied in the perioperative period after median nerve releasing surgery, improves axonal regeneration and muscle reinnervation in carpal tunnel syndrome patients, but without clear improvement in terms of functional outcome [89].

8. Conclusion

Activity-based strategies improve nerve regeneration in rodent models of peripheral nerve injury. Natural stimulation of damaged motor and sensory pathways reinforces intrinsic

neurobiological mechanisms of axonal growth, leading to faster and more complete target reinnervation. Central nervous system plasticity seems to be an important component of functional recovery of complex and adaptive sensorimotor behavior following peripheral nerve injury. Rat models may be useful to identify and develop novel activity-based strategies that stimulate axonal regeneration and central nervous system function, ultimately leading to improved functional outcome following peripheral nerve injury. Translation of such knowledge to clinical practice is desirable, although it must be carried out with caution and taking into consideration the clinical experience.

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References

[1] Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, et al. Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. Journal of the Peripheral Nervous System. 2010;15(2): 120-127.

[2] Lad SP, Nathan JK, Schubert RD, Boakye M. Trends in median, ulnar, radial, and brachioplexus nerve injuries in the United States. Neurosurgery. 2010 May;66(5): 953-960.

[3] Maripuu A, Bjorkman A, Bjorkman-Burtscher I, Mannfolk P, Andersson G, Dahlin L. Reconstruction of sciatic nerve after traumatic injury in humans-factors influencing outcome as related to neurobiological knowledge from animal research. Journal of Brachial Plexus and Peripheral Nerve Injury. 2012;7(1):7; http://www.jbppni.com/content/7/1/7.

[4] Abe N, Cavalli V. Nerve injury signaling. Current Opinion in Neurobiology. 2008 Jun;18(3):276-283.

[5] Chierzi S, Ratto GM, Verma P, Fawcett JW. The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. The European Journal of Neuroscience. 2005 Apr;21(8):2051-2062.

[6] Verma P, Chierzi S, Codd AM, Campbell DS, Meyer RL, Holt CE, et al. Axonal Protein Synthesis and Degradation Are Necessary for Efficient Growth Cone Regeneration. The Journal of Neuroscience. 2005 January 12, 2005;25(2):331-342.

[7] Han Q-J, Gao N-N, Guo-QiangMa, Zhang Z-N, Yu W-H, Pan J, et al. IPP5 inhibits neurite growth in primary sensory neurons by maintaining TGF-β/Smad signaling. Journal of Cell Science. 2013 January 15, 2013;126(2):542-553.

[8] Agthong S, Kaewsema A, Tanomsridejchai N, Chentanez V. Activation of MAPK ERK in peripheral nerve after injury. BMC Neuroscience. 2006;7(1):45; http://www.biomedcentral.com/1471-2202/7/45.

[9] Abe N, Borson SH, Gambello MJ, Wang F, Cavalli V. Mammalian Target of Rapamycin (mTOR) Activation Increases Axonal Growth Capacity of Injured Peripheral Nerves. Journal of Biological Chemistry. 2010 September 3, 2010;285(36):28034-28043.

[10] Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Molecular Neurobiology. 2003 2003/06/01;27(3):277-323.

[11] Boyd JG, Gordon T. The neurotrophin receptors, trkB and p75, differentially regulate motor axonal regeneration. Journal of Neurobiology. 2001;49(4):314-325.

[12] Rotshenker S. Wallerian degeneration: the innate-immune response to traumatic nerve injury. Journal of Neuroinflammation. 2011;8:109; http://www.jneuroinflammation.com/content/8/1/109.

[13] Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. Journal of Neuroinflammation. 2011;8:110; http://www.jneuroinflammation.com/content/8/1/110.

[14] Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. The Journal of Neuroscience. 1995 May;15(5 Pt 2): 3876-3885.

[15] Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. The Journal of Neuroscience. 1995 May;15(5 Pt 2):3886-3895.

[16] Gordon T, Tyreman N, Raji MA. The basis for diminished functional recovery after delayed peripheral nerve repair. The Journal of Neuroscience. 2011 Apr 6;31(14): 5325-5334.

[17] Sulaiman W, Gordon T. Neurobiology of Peripheral Nerve Injury, Regeneration, and Functional Recovery: From Bench Top Research to Bedside Application. The Ochsner Journal. 2013 2013/03/01;13(1):100-108.

[18] Sulaiman OAR, Voda J, Gold BG, Gordon T. FK506 Increases Peripheral Nerve Regeneration after Chronic Axotomy but Not after Chronic Schwann Cell Denervation. Experimental Neurology. 2002;175(1):127-137.

[19] Sulaiman OAR, Midha R, Munro CA, Matsuyama T, Al-Majed A, Gordon T. Chronic Schwann Cell Denervation and the Presence of a Sensory Nerve Reduce Motor Axonal Regeneration. Experimental Neurology. 2002;176(2):342-354.

[20] Sulaiman OA, Gordon T. Role of chronic Schwann cell denervation in poor functional recovery after nerve injuries and experimental strategies to combat it. Neurosurgery. 2009 Oct;65(4 Suppl):A105-114.

[21] Borisov AB, Carlson BM. Cell death in denervated skeletal muscle is distinct from classical apoptosis. The Anatomical Record. 2000 Mar 1;258(3):305-318.

[22] Ijkema-Paassen J, Meek MF, Gramsbergen A. Reinnervation of muscles after transection of the sciatic nerve in adult rats. Muscle Nerve. 2002 Jun;25(6):891-897.

[23] Borisov AB, Dedkov EI, Carlson BM. Abortive myogenesis in denervated skeletal muscle: differentiative properties of satellite cells, their migration, and block of terminal differentiation. Anatomy and Embryology (Berl). 2005 Apr;209(4):269-279.

[24] Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM. Reparative myogenesis in long-term denervated skeletal muscles of adult rats results in a reduction of the satellite cell population. The Anatomical Record. 2001 Jun 1;263(2):139-154.

[25] Brushart TM. Preferential reinnervation of motor nerves by regenerating motor axons. The Journal of Neuroscience. 1988 Mar;8(3):1026-1031.

[26] de Ruiter GC, Malessy MJ, Alaid AO, Spinner RJ, Engelstad JK, Sorenson EJ, et al. Misdirection of regenerating motor axons after nerve injury and repair in the rat sciatic nerve model. Experimental Neurology. 2008 Jun;211(2):339-350.

[27] 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. The Journal of Neuroscience. 2002 Aug 1;22(15):6631-6638.

[28] Al-Majed AA, Neumann CM, Brushart TM, Gordon T. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. The Journal of Neuroscience. 2000 Apr 1;20(7):2602-2608.

[29] English AW. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. Journal of Comparative Neurology. 2005 Oct 3;490(4):427-441.

[30] van Meeteren NLU, Brakkee JH, Hamers FPT, Helders PJM, Gispen WH. Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. Archives of Physical Medicine and Rehabilitation. 1997;78(1):70-77.

[31] English AW, Wilhelm JC, Sabatier MJ. Enhancing recovery from peripheral nerve injury using treadmill training. Annals of Anatomy-Anatomischer Anzeiger. 2011;193(4):354-361.

[32] English AW, Cucoranu D, Mulligan A, Sabatier M. Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. Journal of Comparative Neurology. 2009 Nov 10;517(2):245-255.

[33] Sabatier MJ, Redmon N, Schwartz G, English AW. Treadmill training promotes axon regeneration in injured peripheral nerves. Experimental Neurology. 2008 Jun;211(2): 489-493.

[34] Ilha J, Araujo RT, Malysz T, Hermel EE, Rigon P, Xavier LL, et al. Endurance and resistance exercise training programs elicit specific effects on sciatic nerve regeneration after experimental traumatic lesion in rats. Neurorehabilitation and Neural Repair. 2008 Jul-Aug;22(4):355-366.

[35] Asensio-Pinilla E, Udina E, Jaramillo J, Navarro X. Electrical stimulation combined with exercise increase axonal regeneration after peripheral nerve injury. Experimental Neurology. 2009 Sep;219(1):258-265.

[36] Udina E, Puigdemasa A, Navarro X. Passive and active exercise improve regeneration and muscle reinnervation after peripheral nerve injury in the rat. Muscle Nerve. 2011 Apr;43(4):500-509.

[37] Pachter BR, Eberstein A. Passive exercise and reinnervation of the rat denervated extensor digitorum longus muscle after nerve crush. American Journal of Physical Medicine and Rehabilitation. 1989 Aug;68(4):179-182.

[38] Angelov DN, Ceynowa M, Guntinas-Lichius O, Streppel M, Grosheva M, Kiryakova SI, et al. Mechanical stimulation of paralyzed vibrissal muscles following facial nerve injury in adult rat promotes full recovery of whisking. Neurobiology of Disease. 2007 Apr;26(1):229-242.

[39] Evgenieva E, Schweigert P, Guntinas-Lichius O, Pavlov S, Grosheva M, Angelova S, et al. Manual stimulation of the suprahyoid-sublingual region diminishes polynnervation of the motor endplates and improves recovery of function after hypoglossal nerve injury in rats. Neurorehabilitation and Neural Repair. 2008 Nov-Dec;22(6): 754-768.

[40] Pavlov SP, Grosheva M, Streppel M, Guntinas-Lichius O, Irintchev A, Skouras E, et al. Manually-stimulated recovery of motor function after facial nerve injury requires intact sensory input. Experimental Neurology. 2008 May;211(1):292-300.

[41] Allodi I, Udina E, Navarro X. Specificity of peripheral nerve regeneration: interactions at the axon level. Progress in Neurobiology. 2012 Jul;98(1):16-37.

[42] Vaynman S, Ying Z, Gomez-Pinilla F. Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience. 2003;122(3):647-657.

[43] Funakoshi H, Frisén J, Barbany G, Timmusk T, Zachrisson O, Verge VM, et al. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. The Journal of Cell Biology. 1993 October 15, 1993;123(2):455-465.

[44] Kobayashi NR, Bedard AM, Hincke MT, Tetzlaff W. Increased Expression of BDNF and trkB mRNA in Rat Facial Motoneurons after Axotomy. European Journal of Neuroscience. 1996;8(5):1018-1029.

[45] Boyd JG, Gordon T. A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. European Journal of Neuroscience. 2002;15(4):613-626.

[46] Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. European Journal of Neuroscience. 2000;12(12):4381-4390.

[47] Ying Z, Roy RR, Edgerton VR, Gomez-Pinilla F. Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. Experimental Neurology. 2005 Jun;193(2):411-419.

[48] Gomez-Pinilla F, Ying Z, Opazo P, Roy RR, Edgerton VR. Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle. The European Journal of Neuroscience. 2001 Mar;13(6):1078-1084.

[49] Wilhelm JC, Xu M, Cucoranu D, Chmielewski S, Holmes T, Lau K, et al. Cooperative Roles of BDNF Expression in Neurons and Schwann Cells Are Modulated by Exercise to Facilitate Nerve Regeneration. The Journal of Neuroscience. 2012 April 4, 2012;32(14):5002-5009.

[50] Sohnchen J, Grosheva M, Kiryakova S, Hubbers CU, Sinis N, Skouras E, et al. Recovery of whisking function after manual stimulation of denervated vibrissal muscles requires brain-derived neurotrophic factor and its receptor tyrosine kinase B. Neuroscience. 2010 Sep 29;170(1):372-380.

[51] Sterne GD, Brown RA, Green CJ, Terenghi G. Neurotrophin-3 Delivered Locally via Fibronectin Mats Enhances Peripheral Nerve Regeneration. European Journal of Neuroscience. 1997;9(7):1388-1396.

[52] Sterne GD, Coulton GR, Brown RA, Green CJ, Terenghi G. Neurotrophin-3–enhanced Nerve Regeneration Selectively Improves Recovery of Muscle Fibers Expressing Myosin Heavy Chains 2b. The Journal of Cell Biology. 1997 November 3, 1997;139(3):709-715.

[53] Goldspink G. Mechanical signals, IGF-I gene splicing, and muscle adaptation. Physiology (Bethesda). 2005 Aug;20:232-238.

[54] Hameed M, Lange KHW, Andersen JL, Schjerling P, Kjaer M, Harridge SDR, et al. The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. The Journal of Physiology. 2004 February 15, 2004;555(1):231-240.

[55] Kim B, Leventhal PS, Saltiel AR, Feldman EL. Insulin-like Growth Factor-I-mediated Neurite Outgrowth in Vitro Requires Mitogen-activated Protein Kinase Activation. Journal of Biological Chemistry. 1997 August 22, 1997;272(34):21268-21273.

[56] Sullivan KA, Kim B, Feldman EL. Insulin-Like Growth Factors in the Peripheral Nervous System. Endocrinology. 2008 December 1, 2008;149(12):5963-5971.

[57] Kanje M, Skottner A, Sjo°berg J, Lundborg Gr. Insulin-like growth factor I (IGF-I) stimulates regeneration of the rat sciatic nerve. Brain Research. 1989;486(2):396-398.

[58] Caroni P, Schneider C, Kiefer MC, Zapf J. Role of muscle insulin-like growth factors in nerve sprouting: suppression of terminal sprouting in paralyzed muscle by IGF-binding protein 4. The Journal of Cell Biology. 1994 May 15, 1994;125(4):893-902.

[59] Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating Insulin-Like Growth Factor I Mediates the Protective Effects of Physical Exercise against Brain Insults of Different Etiology and Anatomy. The Journal of Neuroscience. 2001 August 1, 2001;21(15):5678-5684.

[60] Chang H-C, Yang Y-R, Wang PS, Kuo C-H, Wang R-Y. The Neuroprotective Effects of Intramuscular Insulin-Like Growth Factor-I Treatment in Brain Ischemic Rats. PLoS One. 2013;8(5):e64015; http://dx.doi.org/10.1371%2Fjournal.pone.0064015.

[61] Kiryakova S, Sohnchen J, Grosheva M, Schuetz U, Marinova T, Dzhupanova R, 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). Experimental Neurology. 2010 Apr;222(2):226-234.

[62] Griggs RC, Kingston W, Jozefowicz RF, Herr BE, Forbes G, Halliday D. Effect of testosterone on muscle mass and muscle protein synthesis. Journal of Applied Physiology. 1989 Jan;66(1):498-503.

[63] Jones KJ, Brown TJ, Damaser M. Neuroprotective effects of gonadal steroids on regenerating peripheral motoneurons. Brain Research Reviews. 2001;37(1–3):372-382.

[64] Sharma N, Marzo SJ, Jones KJ, Foecking EM. Electrical stimulation and testosterone differentially enhance expression of regeneration-associated genes. Experimental Neurology. 2010;223(1):183-191.

[65] Wood K, Wilhelm JC, Sabatier MJ, Liu K, Gu J, English AW. Sex differences in the effectiveness of treadmill training in enhancing axon regeneration in injured peripheral nerves. Developmental Neurobiology. 2012;72(5):688-698.

[66] Ahtiainen JP, Pakarinen A, Alen M, Kraemer WJ, Hakkinen K. Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. European Journal of Applied Physiology. 2003 Aug;89(6):555-563.

[67] Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T. Outcome measures of peripheral nerve regeneration. Annals of Anatomy. 2011 Jul;193(4):321-333.

[68] Varejao AS, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, et al. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. Journal of Neurotrauma. 2004 Nov;21(11): 1652-1670.

[69] Joao F, Amado S, Veloso A, Armada-da-Silva P, Mauricio AC. Anatomical reference frame versus planar analysis: implications for the kinematics of the rat hindlimb during locomotion. Reviews in the Neurosciences. 2010;21(6):469-485.

[70] Sabatier MJ, To BN, Nicolini J, English AW. Effect of Axon Misdirection on Recovery of Electromyographic Activity and Kinematics after Peripheral Nerve Injury. Cells Tissues Organs. 2011 Mar 17;193(5):298-309.

[71] Howard CS, Blakeney DC, Medige J, Moy OJ, Peimer CA. Functional assessment in the rat by ground reaction forces. Journal of Biomechanics. 2000 Jun;33(6):751-757.

[72] Bennett SW, Lanovaz JL, Muir GD. The biomechanics of locomotor compensation after peripheral nerve lesion in the rat. Behavioral and Brain Research. 2012 Apr 15;229(2):391-400.

[73] Luis AL, Rodrigues JM, Geuna S, Amado S, Shirosaki Y, Lee JM, et al. Use of PLGA 90:10 Scaffolds Enriched with In Vitro-Differentiated Neural Cells for Repairing Rat Sciatic Nerve Defects. Tissue Engineering Part A. 2008 Jun;14(6):979-993.

[74] Amado S, Rodrigues JM, Luis AL, Armada-da-Silva PA, Vieira M, Gartner A, et al. Effects of collagen membranes enriched with in vitro-differentiated N1E-115 cells on rat sciatic nerve regeneration after end-to-end repair. Journal of Neuroengineering and Rehabilitations. 2010;7:7; http://www.jneuroengrehab.com/content/7/1/7.

[75] Amado S, Simoes MJ, Armada da Silva PA, Luis AL, Shirosaki Y, Lopes MA, et al. Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. Biomaterials. 2008 Nov;29(33):4409-4419.

[76] Gartner A, Pereira T, Armada-da-Silva PA, Amorim I, Gomes R, Ribeiro J, et al. Use of poly(DL-lactide-epsilon-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly of the umbilical cord for promoting nerve regeneration in axonotmesis: in vitro and in vivo analysis. Differentiation. 2012 Dec;84(5):355-365.

[77] Amado S, Armada-da-Silva PA, Joao F, Mauricio AC, Luis AL, Simoes MJ, et al. The sensitivity of two-dimensional hindlimb joint kinematics analysis in assessing functional recovery in rats after sciatic nerve crush. Behavioural Brain Research. 2011 Dec 1;225(2):562-573.

[78] Alvarez FJ, Bullinger KL, Titus HE, Nardelli P, Cope TC. Permanent reorganization of Ia afferent synapses on motoneurons after peripheral nerve injuries. Annals of the New York Academy of Sciences. 2010;1198(1):231-241.

[79] Maas H, Prilutsky BI, Nichols TR, Gregor RJ. The effects of self-reinnervation of cat medial and lateral gastrocnemius muscles on hindlimb kinematics in slope walking. Experimental Brain Research. 2007 Aug;181(2):377-393.

[80] Alvarez FJ, Titus-Mitchell HE, Bullinger KL, Kraszpulski M, Nardelli P, Cope TC. Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. I. Loss of VGLUT1/IA synapses on motoneurons. Journal of Neurophysiology. 2011 November 1, 2011;106(5):2450-2470.

[81] Bullinger KL, Nardelli P, Pinter MJ, Alvarez FJ, Cope TC. Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. II. Loss of functional connectivity with motoneurons. Journal of Neurophysiology. 2011 November 1, 2011;106(5):2471-2485.

[82] Chen Y, Wang Y, Chen L, Sun C, English AW, Wolpaw JR, et al. H-reflex up-conditioning encourages recovery of EMG activity and H-reflexes after sciatic nerve transection and repair in rats. The Journal of Neuroscience. 2010 Dec 1;30(48):16128-16136.

[83] Boeltz T, Ireland M, Mathis K, Nicolini J, Poplavski K, Rose SJ, et al. Effects of treadmill training on functional recovery following peripheral nerve injury in rats. Journal of Neurophysiology. 2013 June 1, 2013;109(11):2645-2657.

[84] Page MJ, O'Connor D, Pitt V, Massy-Westropp N. Exercise and mobilisation interventions for carpal tunnel syndrome. Cochrane Database of Systematic Reviews. 2012;6:CD009899.

[85] Caliandro P, La Torre G, Padua R, Giannini F, Padua L. Treatment for ulnar neuropathy at the elbow. Cochrane Database of Systematic Reviews. 2012;7:CD006839.

[86] Teixeira LJ, Valbuza JS, Prado GF. Physical therapy for Bell's palsy (idiopathic facial paralysis). Cochrane Database Syst Rev. 2011(12):CD006283.

[87] White Claire M, Pritchard J, Turner-Stokes L. Exercise for people with peripheral neuropathy. Cochrane Database of Systematic Reviews [serial on the Internet]. 2004; (4).

[88] Beurskens CHG, Burgers-Bots IAL, Kroon DW, Oostendorp RAB. Literature Review of Evidence Based Physiotherapy in Patients with Facial Nerve Paresis. Journal of the Japanese Physical Therapy Association. 2004;7(1):35-39.

[89] Gordon T, Amarjani N, Edwards DC, Chan KM. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. Experimental Neurology. 2009 Oct 1.