Published in final edited form as:

Exerc Sport Sci Rev. 2015 July ; 43(3): 163–171. doi:10.1249/JES.0000000000000047.

Pathways Mediating Activity-Induced Enhancement of Recovery from Peripheral Nerve Injury

Manning J. Sabatier¹ and Arthur W. English²

¹Dept of Rehabilitation Medicine, Emory University School of Medicine, Atlanta GA

²Dept of Cell Biology Emory University School of Medicine, Atlanta GA

Abstract

This paper outlines the novel hypothesis that exercise promotes axon regeneration after peripheral nerve injury through neuronal brain derived neurotrophic factor (BDNF), and there are three required means of promoting BDNF expression: 1) increased signaling through androgen receptors; 2) increased cAMP-responsive element binding protein expression; and 3) increased expression of the transcription factor, SRY-box containing gene 11.

Keywords

treadmill training; axon regeneration; rehabilitation; neurotrophins; BDNF; trkB

INTRODUCTION

Peripheral nerve injury (PNI) is a significant contributor to disability in the United States. Among the 200,000+ victims of new PNI's in the US each year (31), only about 10% ever recover full function and many will seek treatment for pain or some other disability (30). Although axons in peripheral nerves are capable of significant regeneration after PNI, inappropriate target reinnervation and slow elongation of regenerating axons are impediments to functional recovery (16). The distances regenerating axons must elongate can be considerable in humans, further complicating recovery. A number of approaches to improve recovery after PNI have been tested, including improved surgical techniques, grafts and grafting techniques, and enzyme treatments to clear the way for axon regeneration (28). However, there is currently no effective treatment that reliably results in full return of function.

A number of studies have shown that moderate exercise incorporating the affected limb enhances axon regeneration after PNI. Treadmill exercise has been used as a model of natural neural activation and to exert experimental control over exercise volume and intensity. These studies have utilized rodent models extensively in order to standardize the injury and, at the same time, examine the biological mechanisms that explain the exercise

effect. This use of animal models of PNI also has made it possible to study the neurophysiology and biomechanics of recovery concurrently. This review provides a brief examination of the literature and concludes that there are several cellular/molecular mechanisms that most likely explain the effect of exercise on recovery from PNI. The following novel hypothesis is proposed: exercise promotes axon regeneration after PNI through neuronal brain derived neurotrophic factor (BDNF), and there are three required means of promoting BDNF expression: 1) increased signaling through androgen receptors; 2) increased Ca^{2+} , cyclic AMP resulting in increased cAMP-responsive element binding protein (CREB) expression; and 3) increased expression of the transcription factor, SRY-box containing gene 11 (Sox11).

EXERCISE ENHANCES AXON REGENERATION AFTER PNI

Data from studies of electrical stimulation, neurotrophins and peripheral nerve injury initially encouraged speculation that exercise might improve recovery from PNI. For instance, Gordon and colleagues found that both motor (2) and sensory (13) axon regeneration was enhanced by as little as one hour of continuous (20 Hz) supramaximal electrical stimulation (ES) of the proximal stump of a cut nerve. Blocking propagation of the evoked action potentials from reaching the cell bodies of these neurons by injecting the sodium channel blocker, tetrodotoxin, into the nerve proximal to the stimulation site resulted in a complete loss of the enhancement induced by ES (2). This finding has been interpreted to mean that for enhanced regeneration to occur, the axon must have been stimulated directly by the treatment. If artificial activation of neurons by ES evokes an increase in axon regeneration, then natural activation of populations of axons by using exercise might increase axon regeneration as well. Increased activity in both sensory neurons and motoneurons is well established with ES. However, there may be differences in the way motoneurons and sensory neurons respond to natural activation with exercise. The connection between exercise, neural recruitment, and axon regeneration is supported indirectly by experimental evidence (detailed in this manuscript). However, activity during treadmill training of motoneurons whose axons have been and are regenerating has not yet been studied directly. Nevertheless, a major reason compelling the investigation of exercise is that it empowers the patient to facilitate his rehabilitation. Therefore, treadmill exercise was chosen as a starting point in our lab because it can be used both by laboratory animals and by human subjects. We expect the results obtained from animal studies of treadmill exercise to have better potential for seamless transition to human subjects.

Exercise training characterized by repetitive movements involving major muscle groups and little or no added resistance has usually been used to study the effect of exercise on recovery from PNI. Although these exercise programs have not been designed to improve endurance or health per se, they nevertheless are most similar to traditional endurance training that is low or moderate intensity. Volume and intensity are the two major defining components of an endurance exercise program that determine the ensuing physiological adaptations. Volume generally refers to how long the exercise task is performed. More volume could be applied by increasing either the frequency (e.g., sessions per week) or the duration of exercise bouts, or both. Intensity generally refers to how much energy was expended per unit time during exercise and is often manipulated by changing the speed of performance of

the task. Volume and intensity are inversely related in exercise prescription. For instance, less exercise volume is required to elicit physiological adaptations when exercise intensity is higher. In clinical studies using endurance exercise with human participants, continuous treadmill locomotion at a slow to moderate pace is commonly used. However, when allowed to exercise voluntarily rodents use a different approach. This approach is characterized by repeated short-duration runs (2 min) at faster speeds, with rest periods interspersed between runs (9), a form of interval training.

We capitalized on this knowledge in our first exploration of the impact of exercise on PNI using mice. We used an interval training (IT) group of mice who ran at a fast speed (20 m/min) for 2 min at a time, separated by 5-min rest periods (26). We trained another group with one hour of continuous locomotion at a slow treadmill speed of 10 m/min (continuous training, CT). Both groups trained five days per week. Training began three days after common fibular nerve (CFN) transection and repair in *thy-1-YFP-H* mouse hosts and lasted two weeks. In these mice a subset of axons in peripheral nerves express yellow fluorescent protein (YFP), enabling visualization of individual axons using fluorescence microscopy. Grafts from non-fluorescent littermates were used to repair the cut nerves because they served as a dark background against which regenerating YFP+ axons could be visualized and measured (Figure 1). This model allows for evaluation of the total number of regenerating axons, and the lengths of those regenerating axons.

Continuous training for longer time periods at slower speeds and interval training for short time periods (as little as 4 min) at higher speeds both resulted in strikingly increased axon elongation at two weeks after nerve transection and repair (Figure 1, inset). Interval training at the slow treadmill speed did not enhance axon elongation. The application of higher intensity with lower volume makes low-volume intermittent exercise (IT) effective at causing statistically significant axon elongation. These results were interpreted to mean that very little exercise volume is required to encourage axon elongation after PNI if exercise intensity is high. Future studies will be needed to determine the threshold for both volume and intensity. Both training effects were confirmed in a follow-up study using the retrograde labeling technique to evaluate the participation of neurons in regeneration (Figure 2, (11)). This technique allows for evaluation of the number of motoneurons whose axons had elongated the distance between the site of nerve transection and the site of retrograde tracer application (5 mm in (11)). It also allows for evaluation of locations of the corresponding cell bodies of these motoneurons in the spinal cord (described in more detail in the following paragraph). We found an increase in the number of motoneurons whose regenerating axons had grown to the site of retrograde tracer application by either 2 or 4 weeks following sciatic nerve transection. Therefore, treadmill training after PNI results in improved axon regeneration irrespective of the experimental outcome measure (i.e., the lengths of regenerating axons, or participation of motoneurons).

One impediment to attaining functional recovery after a PNI might be the reinnervation of functionally inappropriate muscles by regenerating axons. For example, following recovery from injury to a mixed nerve, motor (or sensory) axons that had innervated flexor muscles could be misdirected and reinnervate extensor muscles. We have evaluated the extent of such misdirection in a mouse model of sciatic nerve transection and repair by exploiting the

natural topographic organization of motoneurons in the mouse spinal cord innervating different muscles (10). Results from experiments that evaluated misdirection two weeks after such an injury are illustrated in Figure 2. In intact mice, motoneurons innervating targets of the CFN are restricted in their location to the rostral 60% of the sciatic motor nucleus (Figure 2C, Intact). Axons of motoneurons located in the caudal 40% of the sciatic motor nucleus are found exclusively in the tibial branch of the sciatic nerve. The locations of these cell bodies were evaluated by applying different retrograde fluorescent tracers (i.e., with different colors) to the cut proximal stumps of the tibial and CFNs (Figure 2A), the primary conduits for ankle extensor and ankle flexor motor axons, respectively. The application procedure was performed two weeks after transection and repair of the sciatic nerve in mice.

Very few axons reached the site of retrograde tracer application in untreated mice by 2 weeks (Figure 2C). If axon regeneration is enhanced (e.g., with ES applied at the time of the transection and repair) the cell bodies of many of the motoneurons with axons in the CFN two weeks later are no longer restricted to the rostral 60% of the sciatic motor nucleus, as they are in intact animals. Instead, many motoneuron cell bodies are found in topographically inappropriate locations in the spinal cord, regions of the sciatic motor nucleus containing axons of motoneurons that had been found in the tibial branch of the sciatic nerve exclusively (10). We found that as many as half of the regenerating axons in the CFN originated from motoneurons which had innervated targets of the tibial branch of the nerve prior to PNI. A similar trend is found after CT and IT (11) (Figure 2C). When mice are trained for two weeks as described above (using both CT and IT paradigms), but the application procedure is performed four weeks after transection and repair of the sciatic nerve, the loss of topographic specificity of motoneurons whose axons had regenerated into the CFN is no greater than that found in control mice that are not trained (11.2 ± 2.7 , mean $\pm$ SEM, topographically inappropriate for control mice versus 8.3 ± 2.2 and 13.2 ± 4.8 for CT and IT trained mice, respectively) (11). Moreover, the loss of topographic specificity is significantly less than observed with ES (10, 11). Therefore, exercise invigorates axon regeneration without exacerbating motor reinnervation inaccuracy.

We also observed that even though the lengths of regenerating axons are greater in both CT and IT animals than in untrained controls (26), an increase in the number of motoneurons whose regenerating axons enter the common fibular branch of the sciatic nerve only occurs in IT mice (11). Based on this finding, we hypothesized that there is recruitment of more common fibular motoneurons during exercise by the higher intensity IT than by the larger volume CT. We further hypothesized that this facilitated the enhancement of motor axon regeneration by involving more motoneurons in the regeneration process. The amount of activation encountered during CT might be sufficient to promote motor axon elongation, but only in a more limited number of motoneurons.

To evaluate these hypotheses we studied the effect of upslope CT. When rodents walk on an upwardly inclined ($+20^\circ$) treadmill, the amplitude of electromyographic (EMG) activity in the tibialis anterior (a target of motor axons in the CFN) is increased as compared to when they walk on a level treadmill (27). Therefore, recruitment of motoneurons whose axons project through the CFN into activity would be expected to increase during upslope walking.

Two weeks after injury, axons of more than twice as many motoneurons were found to have regenerated into the CFN of upslope CT mice (84.3 ± 16.1) compared to level CT mice (39.0 ± 8.6) a number comparable to that observed in level IT mice (75.0 ± 8.7) (25) (Figure 2C). These results are consistent with the hypothesis that treadmill training results in an enhancement of axon regeneration by natural recruitment of motoneurons via the spinal circuits that drive locomotion. We also found that more regenerating motor axons were misdirected after upslope training. Therefore, changing the biomechanical demands of exercise could be used to direct the enhancement of the regeneration of different groups of motor axons.

NEUROTROPHINS ARE REQUIRED FOR THE ENHANCING EFFECT OF EXERCISE

How might exercise, or increased neuronal activity, be transduced into what is known about the requirements for axon regeneration at a cellular or even molecular level? The novel hypothesis proposed in this review synthesizes the major signaling pathways thought to be involved and is illustrated in Figure 3. The major elements of Figure 3 are explained in the following sections. Regenerating neurites from the proximal segment of a cut nerve must enter a pathway in the distal segment of the nerve if axons are to regenerate and reinnervate their targets. Among the molecules that they encounter in that pathway is brain derived neurotrophic factor (BDNF), which is produced by transformed Schwann cells and promotes axon elongation by binding to trkB receptors on the regenerating axons (12). Once regenerating axons have contacted these transformed Schwann cells, they cease to secrete BDNF and commence in remyelination.

In our mouse model of PNI, we found that if we constrained regenerating axons to grow into a pathway in which the gene for BDNF had been knocked out in Schwann cells, very little elongation occurred, but if we treadmill trained these mice, elongation was enhanced (Figure 4) (33). Increased axon elongation occurred regardless of the neurotrophin content of the pathway into which the axons regenerated. However, neurons, especially motoneurons, also synthesize and secrete BDNF. Electrical stimulation (3), as well as both voluntary exercise (14) and treadmill training (22, 33), have been shown to result in increased expression of BDNF mRNA in motoneurons. If the gene for BDNF was knocked out selectively in neurons, then the effects of exercise on axon elongation were lost (33) (Figure 4). From these data we inferred that neuron-derived BDNF, acting in an autocrine manner, mediates the effect of treadmill training on elongation of regenerating axons after PNI. We believe the results referenced above are consistent with the idea that increased BDNF expression requires increased neuronal activity and that increased signaling via neuron-derived BDNF is required for the enhancement of axon regeneration in peripheral nerves produced by exercise (1, 33).

There are several pathways through which neuronal activity may increase BDNF synthesis. Expression of the BDNF gene is regulated by multiple promoters in a tissue-, context-, and activity-dependent manner. As many as seventeen different BDNF mRNAs, each encoding the same protein, have been found. The different mRNAs are formed from differential splicing of the multiple 3' non-coding exons. The calcium response element, cAMP

response element-binding protein (CREB), has been identified as an important synaptic activity-regulated transcription factor (17). It selectively transduces the activation of calcium signaling pathways into BDNF exon IV transcription. We believe the SRY-box containing gene 11 (Sox11), which induces BDNF gene transcription via activity of mRNAs containing exon 1 of the BDNF gene (Figure 3), may be part of another important pathway for transducing the effect of exercise into BDNF synthesis and therefore axon regeneration. Expression of both exon 1-containing mRNA, and the Sox11 transcription factor are increased following axotomy (29). In fact, Salerno and colleagues found that of ten BDNF 3' exons evaluated after axotomy, mRNAs containing exon 1 increased the most (29). If Sox11 expression is knocked down by injection of small interfering RNAs (siRNAs) into the mouse saphenous nerve, axon regeneration after nerve injury is transiently inhibited (18). Moreover, in one of the first studies to evaluate the effect of exercise on BDNF, the Cotman laboratory showed that voluntary wheel running enhanced exon I-containing mRNA expression (23). Therefore, future studies should test the hypothesis that Sox11 activation is a significant factor in transducing neural activity into an increase in BDNF synthesis and enhanced axon regeneration.

THE EFFECT OF EXERCISE ON RECOVERY FROM PNI DEPENDS ON ANDROGENS

It is now well known that androgens play an important role in recovery following PNI. For instance, treatment with testosterone has been shown to promote recovery in models of sciatic nerve crush, facial nerve crush and recurrent laryngeal injury (reviewed in (21)). Based on this knowledge, one could speculate that the markedly different levels of endogenous androgens in males and females might lead to differences in the response to exercise. In fact, in the course of evaluating the effect of exercise on elongation of regenerating axons in mice, we discovered a marked sex difference. In male mice, one hour of daily slow CT results in a marked increase in the length of regenerating axons two weeks later, but the very same exercise protocol has no enhancing effect in females. In female mice exposed to daily IT the enhancement on axon elongation is impressive, but no enhancement is found in male mice exposed to the same IT protocol (34). Castrating males blocked the enhancing effect of exercise completely and did not influence the ineffectiveness of the IT protocol. Treatments of unexercised female mice with an inhibitor of P450 aromatase, an enzyme which catalyzes the conversion of testosterone and its precursors into estradiol, resulted in a striking enhancement of the lengths of regenerating axons (34). In subsequent experiments, we found that treating mice with the androgen receptor blocker, flutamide, blocked the effect of both exercise and electrical stimulation in both males and females (32). We interpreted these results to mean that signaling through androgen receptors is necessary for the enhancing effects of treadmill exercise on axon elongation after PNI, irrespective of sex.

The interaction of sex and exercise extends beyond peripheral axon regeneration to central spinal synaptic connections involving the affected neurons. Changes in the central circuitry have received relatively little attention as a contributor to poor functional recovery after PNI. However, after PNI there is a significant withdrawal of synaptic inputs from the somata

and proximal dendrites of axotomized motoneurons (4). Therefore, in a subsequent study we evaluated the coverage of contacts made by two different types of synapses onto motoneurons following peripheral nerve transection: 1) excitatory contacts made mainly by primary afferent neurons which contain vesicular glutamate transporter 1 (VGLUT1), and 2) inhibitory contacts containing glutamic acid decarboxylase (GAD67) arising from within the central nervous system. If both male and female mice were appropriately exercised following injury, the anticipated reduction in synaptic coverage on the axotomized motoneurons by both of these types of synapses (as shown in Figure 5) was not found (21). We also found that this effect of exercise is blocked completely by flutamide treatments in both males and females (21). Therefore, exercise has similar effects on synaptic coverage of axotomized motoneurons in male and female mice, but the requirements for exercise to produce these effects are different in male and female mice. Moreover, cellular signaling through the androgen receptor is required for the effects of exercise in both sexes, whether those effects are peripheral axon regeneration or maintenance of contacts made by synaptic inputs with the somata of motoneurons.

A UNIFYING HYPOTHESIS

The novel hypothesis proposed in this review is based on our own data and on review of the literature and centers on the importance of neuronal BDNF in mediating the effect of exercise (Figure 3). There are at least three ways that the increased activity associated with exercise could result in increased expression of neuronal BDNF, each centered around the generation of different BDNF mRNAs. Increased neuronal activity induced by exercise could increase the availability of androgens to neurons whose axons are regenerating. Production of BDNF is known to be downstream from motoneuron androgen receptor signaling, associated with mRNAs containing exon VI. Blocking androgen receptor signaling blocks the effects of increased neuronal activity in promoting axon regeneration (32). Increased neuronal activity results in increased calcium influx and increased production of CREB. Exon IV-containing BDNF mRNA is the major form induced by increased neuronal activity. In mice in which the CREB binding domain of exon IV is mutated, activity-dependent BDNF expression is reduced dramatically (17). Increased activity also results in increased expression of exon I-containing BDNF mRNA (23), whereas knockdown of the exon-I promoting transcription factor, Sox11, inhibits axon regeneration (29). Thus our unifying hypothesis is that all three pathways are required for activity-associated enhancement of axon regeneration.

This hypothesis must be considered a working hypothesis. In future studies, new regulatory mechanisms may be discovered that render this model overly simplistic, but we feel that our hypothesis has immediate value because it can be tested experimentally, especially at the points indicated in Figure 3. In addition, we recognize that our hypothesis does not consider whether the proposed mechanism is cell autonomous. We assume that the site of the androgen receptor signaling required for enhancing axon regeneration using activity-based treatments is the neurons whose axons are regenerating. However, androgens also might act on other cell types. For example, androgen receptor immunoreactivity has been described on astrocytes and microglia, especially after injury. It is possible that non-neuronal androgen receptor signaling could initiate an intercellular signaling mechanism that results in

increased expression of BDNF in neurons whose axons are regenerating. A second advantage of our unifying hypothesis is that it could be modified readily to include more complex cell-cell interactions and retain its testability.

THE EFFECTS OF EXERCISE EXTEND BEYOND AXON REGENERATION TO FUNCTIONAL RECOVERY

The maintenance of excitatory, VGLUT1+, motoneuronal synaptic coverage with exercise may have important implications for regaining normal movement capabilities. For instance, the muscle stretch reflex is a fundamental unit of proprioception that is required for normal movement. Stretch sensitive afferents synapse with motoneurons at terminals expressing VGLUT1. Encoding of muscle stretch by regenerated proprioceptive afferents after PNI approaches normal, but stretch of a reinnervated muscle fails to produce either reflex contraction or synaptic excitation in most motoneurons (15). In the study cited above (4), the authors also found that PNI results in a permanent loss of VGLUT1 synapses onto motoneurons, and this is thought to be the basis for the permanent loss of the stretch reflex in reinnervated muscle after PNI. Therefore, the maintenance of VGLUT1 spinal neuronal synaptic coverage with exercise could have major positive implications for functional recovery because it suggests that the stretch reflex might be maintained after PNI if exercise is used. This also supports the hypothesis that if exercise results in maintenance of synaptic coverage by VGLUT1 synapses after PNI as we have reported, then functional recovery will be improved as well.

To begin a focus on functional recovery after PNI we investigated the effect of CT treadmill exercise on the compound muscle action potential (M-response) and the muscle potential evoked in response to electrical stimulation of stretch sensitive afferents (H-reflex); and locomotor EMG activity and kinematics. If CT treadmill exercise was used in rats after sciatic nerve transection and repair, not only did H-reflexes and M-responses return earlier, the amplitude of the H-reflex was fully restored (6). Moreover, soleus EMG activity timing during walking at upslope, level and downslope returned to normal; and hindlimb kinematics during level and upslope walking were improved. Therefore, not only is moderate daily exercise sufficient to promote axon regeneration, it also positively impacts spinal neural circuitry and improves the ability of rats to cope with different biomechanical demands of slope walking. Invigorating axon regeneration and preventing the loss of excitatory, VGLUT1, spinal neuronal synaptic coverage with exercise contributes to better functional recovery.

Other evidence from both spinal cord injury and PNI supports the idea that exercise improves functional recovery and this effect is linked to the ability to increase BDNF. For instance, Cote and colleagues showed that recovery of H-reflex frequency-dependent depression in rats was correlated with the increase in BDNF resulting from step- and bike-training (8). Improved functional recovery after PNI involving the forelimb in mice was recently reported from the Hoke laboratory (24). Not only did CT treadmill exercise like ours improve recovery of forelimb grip function, the improvement was also correlated with an increase in BDNF in both the nerves and the serum. These results help to confirm the

effect of exercise on BDNF and suggest that improvements in functional recovery with exercise require an increase in neuronal BDNF.

WHAT DO THESE RESULTS MEAN FOR PEOPLE WITH PNI's?

It is clear from the information reviewed above that the effect of exercise on recovery after PNI requires BDNF. For that reason, it will be important to understand how single nucleotide polymorphisms (SNPs) in the BDNF gene in humans impact the effect of exercise. Several SNPs in the BDNF gene have been described, but the best known of these (rs6265) results in a single amino acid (Val66Met) substitution and is found in more than 25% of the human population (5). Activity-dependent release of BDNF is impaired in cells containing the Val66Met SNP (7), and in a mouse model of this SNP, the mutant BDNF prodomain stimulated growth cone retraction via the common neurotrophin receptor, p75NTR (5). Thus, individuals with the Val66Met SNP might not be able to respond appropriately to activity-based therapies for PNI and their application might even result in an inhibition of axon regeneration.

Indeed, there is evidence in humans that the presence of the Val66Met SNP diminishes neural plasticity. Anodal transcutaneous spinal direct current stimulation (tsDCS) is a noninvasive technique that induces plasticity of spinal neuronal circuits. Fifteen minutes of tsDCS leads to an acute leftward shift of the H reflex recruitment curve in people without the Val66Met SNP, but there is no such shift in those with the Val66Met SNP (20). The Val66Met SNP has also been found to diminish activity-dependent cortical plasticity in humans (19). The implication of these findings is that people with the Val66Met SNP might not respond to exercise training that is thought to improve recovery from PNI. For example, if the correlations found between functional improvement and increased BDNF found in rodent models (for PNI (24) and for SCI (8)) hold up for humans, then we would expect a diminished or null effect of exercise after PNI for people with the Val66Met SNP. Therefore, the parameters of rehabilitation exercise that improve functional recovery in these people might be drastically different.

SUMMARY AND FUTURE DIRECTIONS

Although there remains some misconception that exercise is harmful in the aftermath of neural injury, it is increasingly clear that exercise can be applied safely and effectively. For example, exercise is necessary and well tolerated by stroke survivors and people with spinal cord injury (35). Like stroke and spinal cord injury, PNI reduces the ability to call on muscles to produce movement. However, the evidence presented here suggests it is possible to capitalize on the remaining functional ability to encourage recovery from PNI, and also highlights some of the principles that can form a basis for developing individualized rehabilitation exercise programs. Furthermore, we view the data presented here as support for the use of exercise as a way to train the nervous system, rather than to improve one or more specific components of fitness. Future studies should be conducted to determine how to optimize the exercise rehabilitation plan to generate the most potent effect on recovery from PNI, particularly in the face of genetic liabilities that limit the effect of exercise on BDNF.

Acknowledgements

This work was supported by grants HD032571, HD053669 and USPHS NIH Institutional Research and Academic Career Development grant (K12 GM00680-05). The authors wish to recognize the work of other researchers who could not be cited due to the journal's reference limitations.

References

1. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. *The European journal of neuroscience*. 2000; 12(12):4381–90. [PubMed: 11122348]
2. 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 : the official journal of the Society for Neuroscience*. 2000; 20(7):2602–8. [PubMed: 10729340]
3. 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(3):379–402. [PubMed: 15206821]
4. 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; 106(5):2450–70. [PubMed: 21832035]
5. Anastasia A, Deinhardt K, Chao MV, et al. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nature communications*. 2013; 4:2490.
6. Boeltz T, Ireland M, Mathis K, et al. Effects of Treadmill Training on Functional Recovery Following Peripheral Nerve Injury in Rats. *Journal of neurophysiology*. 2013
7. Chen ZY, Patel PD, Sant G, et al. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2004; 24(18):4401–11. [PubMed: 15128854]
8. Cote MP, Azzam GA, Lemay MA, Zhukareva V, Houle JD. Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury. *Journal of neurotrauma*. 2011; 28(2):299–309. [PubMed: 21083432]
9. De Bono JP, Adlam D, Paterson DJ, Channon KM. Novel quantitative phenotypes of exercise training in mouse models. *American journal of physiology. Regulatory, integrative and comparative physiology*. 2006; 290(4):R926–34.
10. English AW. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *The Journal of comparative neurology*. 2005; 490(4):427–41. [PubMed: 16127712]
11. English AW, Cucoranu D, Mulligan A, Sabatier M. Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. *The Journal of comparative neurology*. 2009; 517(2):245–55. [PubMed: 19731339]
12. Funakoshi H, Frisen J, Barbany G, et al. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J Cell Biol*. 1993; 123(2):455–65. [PubMed: 8408225]
13. Geremia NM, Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. *Experimental neurology*. 2007; 205(2):347–59. [PubMed: 17428474]
14. Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *Journal of neurophysiology*. 2002; 88(5): 2187–95. [PubMed: 12424260]
15. Haftel VK, Bichler EK, Wang QB, Prather JF, Pinter MJ, Cope TC. Central suppression of regenerated proprioceptive afferents. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005; 25(19):4733–42. [PubMed: 15888649]

16. Hoke A. Proteoglycans in axonal regeneration. *Experimental neurology*. 2005; 195(2):273–7. [PubMed: 16040028]
17. Hong EJ, McCord AE, Greenberg ME. A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. *Neuron*. 2008; 60(4): 610–24. [PubMed: 19038219]
18. Jankowski MP, McIlwrath SL, Jing X, et al. Sox11 transcription factor modulates peripheral nerve regeneration in adult mice. *Brain research*. 2009; 1256:43–54. [PubMed: 19133245]
19. Kleim JA, Chan S, Pringle E, et al. BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. *Nat Neurosci*. 2006; 9(6):735–7. [PubMed: 16680163]
20. Lamy JC, Boakye M. BDNF Val66Met polymorphism alters spinal DC stimulation-induced plasticity in humans. *Journal of neurophysiology*. 2013; 110(1):109–16. [PubMed: 23576701]
21. Liu C, Ward PJ, English AW. The effects of exercise on synaptic stripping require androgen receptor signaling. *PloS one*. 2014; 9(6):e98633. [PubMed: 24887087]
22. Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain research*. 1996; 726(1-2): 49–56. [PubMed: 8836544]
23. Oliff HS, Berchtold NC, Isackson P, Cotman CW. Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Brain research. Molecular brain research*. 1998; 61(1-2):147–53. [PubMed: 9795193]
24. Park JS, Hoke A. Treadmill exercise induced functional recovery after peripheral nerve repair is associated with increased levels of neurotrophic factors. *PloS one*. 2014; 9(3):e90245. [PubMed: 24618564]
25. Sabatier, MJ.; Kaufman, M.; English, AW. Effects of Upslope Treadmill Exercise On Axon Regeneration in Peripheral Nerves.. American College of Sports Medicine; Annual Meeting; Denver. 2010. p. CO2010
26. Sabatier MJ, Redmon N, Schwartz G, English AW. Treadmill training promotes axon regeneration in injured peripheral nerves. *Experimental neurology*. 2008; 211(2):489–93. [PubMed: 18420199]
27. Sabatier MJ, To BN, Nicolini J, English AW. Effect of slope and sciatic nerve injury on ankle muscle recruitment and hindlimb kinematics during walking in the rat. *The Journal of experimental biology*. 2011; 214:1007–16. [PubMed: 21346129]
28. Sabatier MJ, To BN, Rose S, Nicolini J, English AW. Chondroitinase ABC reduces time to muscle reinnervation and improves functional recovery after sciatic nerve transection in rats. *Journal of neurophysiology*. 2012; 107(3):747–57. [PubMed: 22049333]
29. Salerno KM, Jing X, Diges CM, Cornuet PK, Glorioso JC, Albers KM. Sox11 modulates brain-derived neurotrophic factor expression in an exon promoter-specific manner. *Journal of neuroscience research*. 2012; 90(5):1011–9. [PubMed: 22331573]
30. Scholz T, Krichevsky A, Sumarto A, et al. Peripheral nerve injuries: an international survey of current treatments and future perspectives. *J Reconstr Microsurg*. 2009; 25(6):339–44. [PubMed: 19301234]
31. Taylor CA, Braza D, Rice JB, Dillingham T. The incidence of peripheral nerve injury in extremity trauma. *American journal of physical medicine & rehabilitation / Association of Academic Physiatrists*. 2008; 87(5):381–5. [PubMed: 18334923]
32. Thompson NJ, Sengelaub DR, English AW. Enhancement of peripheral nerve regeneration due to treadmill training and electrical stimulation is dependent on androgen receptor signaling. *Developmental neurobiology*. 2013
33. Wilhelm JC, Xu M, Cucoranu D, et al. Cooperative roles of BDNF expression in neurons and Schwann cells are modulated by exercise to facilitate nerve regeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32(14):5002–9. [PubMed: 22492055]
34. 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):688–98. [PubMed: 21805686]

35. Zehr EP. Evidence-based risk assessment and recommendations for physical activity clearance: stroke and spinal cord injury. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2011; 36(Suppl 1):S214–31.

Figure 1. Axon regeneration after treadmill training

Low magnification images of two common fibular nerves are displayed to show the effect of treadmill training on the regeneration of cut axons [Adapted from (26). Copyright © 2008 Elsevier. Used with permission.] Each nerve had been cut and surgically repaired two weeks earlier using a ca. 3 mm graft from a wild type donor mouse. The boundaries of each graft are indicated by arrows. The overall image of each nerve is a montage constructed from images of several microscope fields, each taken at the same depth through whole mounts of the nerve. By two weeks after nerve repair, any residual fluorescence in the original distal stump (distal to the graft) due to the degeneration of the host axons has disappeared. Fluorescence found in this region thus represents profiles of regenerating axons that have grown entirely through the grafts. The nerve on the right came from a mouse that had received continuous treadmill training after the nerve repair. The nerve on the left was from a mouse that was unexercised. Average median axon profile lengths (+SEM) from mice exercised with IT (Interval) and CT (Continuous) are expressed as multiples of the average median axon profile length from untreated mice in the graph inset.

Figure 2. Misdirection of axon regeneration after PNI

The experimental protocol is shown in cartoon form in A. The distal sciatic nerve was cut and repaired with fibrin glue just proximal to the branching point of the sural nerve. Two weeks afterward, the two major terminal branches of the sciatic nerve were cut, and retrograde fluorescent tracers were applied to their proximal stumps. These were dextrans conjugated either to Alexafluor 488 (FD488, green) or to Alexafluor 594 (FD594, red). In B is an image taken from a histological section containing retrogradely labeled motoneurons in the ventral horn of the spinal cord of an injured mouse. The dashed line indicates the outline of the gray matter. Cells marked by green fluorescence were labeled by the application of FD488 to the common fibular nerve. Red cells were marked by the application of FD594 to the tibial nerve. In intact mice there is spatial separation of tibial and common fibular motoneuron cell bodies, i.e., cell bodies of axons projecting through the common fibular nerve (green) tend to be found lateral to cell bodies of axons projecting through the tibial nerve (red). In this image, one cell body is double-labeled (white arrow), suggesting axonal branching through both nerves, and a green cell body is found medial to red cell bodies. In C are histograms depicting the relative caudorostral distributions of motoneurons labeled after application of tracer to the common fibular nerve for Intact mice, and for several treatment groups of injured mice at two weeks after transection and surgical repair of the distal sciatic nerve. The most caudal cells in the sciatic motor nucleus are found at location zero. The most rostral cells are found at location 100. In each graph, the numbers to the right of the group designation are 1) left, the mean number of common fibular cell bodies in the rostral 40% of the distribution where only tibial cell bodies are normally found, and 2) right, the mean total number of common fibular labeled cells. The dashed line in the graph on the left is placed at the 40% mark.

Figure 3. Neural activity prompts a cascade of events that promote enhanced axon regeneration

The flow-chart in fig 3 illustrates the novel hypothesis of this review. Exercise-related increased neural activity could affect axon regeneration in three ways. 1) The first is to increase androgen receptor signaling. Data support the idea that this is accomplished in two ways. 1a) The first is to decrease P450 aromatase activity (thereby increasing testosterone because less is converted to estradiol). This route primarily occurs in female mice as a result of interval training (IT) at a higher treadmill speed, 20 m/min. 1b) The second of the two proposed routes of androgen signaling is via increased 5 α reductase activity (thereby increasing the rate of conversion of testosterone to dihydrotestosterone (DHT), the primary androgen that mediates the downstream biological effects resulting in better axon regeneration). This route primarily occurs in male mice as a result of continuous training (CT) at a lower treadmill speed, 10 m/min. DHT and testosterone signaling through the androgen receptor (AR) results in increased BDNF expression via generation of mRNA's containing exon VI of the BDNF gene. 2) The second way that exercise-related increased neural activity could affect axon regeneration is to increase cAMP-responsive element binding protein (CREB) expression. CREB activation of exon IV, of the BDNF gene increases BDNF gene transcription. 3) The third way is to increase expression of the SRY-box containing gene 11, Sox11, expression. Sox11 activation of exon I of the BDNF gene increases BDNF gene transcription. BDNF promotes axon regeneration by autocrine signaling through the trkB receptor. Our hypothesis predicts that all three pathways are required.

Figure 4. The enhancing effect of exercise on axon regeneration after PNI is dependent on BDNF in regenerating axons

Median axon profile lengths of a wild-type (WT) unexercised group, and several transgenic mouse/exercise condition groups are expressed as a ratio to the average median axon profile length of an untreated WT control group. Error bars are 95% confidence intervals. All groups were significantly different from the untreated WT group. Plus signs (+) beneath the x-axis mean that mice from that group were exercised with treadmill training. Regenerating axons from exercised WT mice with grafts from WT donors had the longest median axon profile lengths. In these experiments, for each sex, the training paradigm that has been shown to enhance axon regeneration was used. Thus, male mice were trained continuously for 1 h at a slow speed of 10 m/min (continuous training) and female mice performed four repetitions at a faster speed of 20 m/min with a 5 min rest period (interval training). The lengths of regenerating axons from WT mice whose nerves were repaired with grafts lacking BDNF (Schwann cell KO) were significantly decreased. In such mice, if exercise was used, there was an enhancement of axon regeneration despite the lack of Schwann cell-derived BDNF. The lengths of regenerating axons from mice with the gene for BDNF knocked out selectively in neurons, and whose nerves were repaired with grafts lacking BDNF (Schwann cell & neuron KO) were significantly decreased. In such mice exercise did not improve axon regeneration.

Figure 5. Synaptic stripping after sciatic nerve transection

Two representative retrogradely labeled motoneurons (red) are shown to demonstrate the reduction in coverage of excitatory and inhibitory synapses on the axotomized motoneurons. These images are from histological sections reacted with an antibody to glutamic acid decarboxylase 67 (GAD67, green) and another to vesicular glutamate transporter 1 (VGLUT1, blue). A region of interest (ROI) around the perimeter of this profile of the motoneuron was created (not shown here) and the green and blue fluorescence intensities were measured beneath the ROI. In the resulting plot profiles (bottom), positive and negative deflections correspond with contacts of the motoneuron soma and structures immunoreactive for synapse-specific antigens for VGLUT1 (positive deflections, blue) and GAD67 (negative deflections, green). The percent coverage of the motoneuron soma perimeter with each synapse-specific antigen is included with each respective trace. The percent coverage of synapse-specific antigen was reduced at 10 weeks post transection.