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Arthur W. English, Jennifer C. Wilhelm and Patricia J. Ward
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Exercise, Neurotrophins, and Axon Regeneration in the PNS

Electrical stimulation and exercise are treatments to enhance recovery from peripheral nerve injuries. Brain-derived neurotrophic factor and androgen receptor signaling are requirements for the effectiveness of these treatments. Increased neuronal activity is adequate to promote regeneration in injured nerves, but the dosing of activity and its relationship to neurotrophins and sex steroid hormones is less clear. Translation of these therapies will require principles associated with their cellular mechanisms.

Following traumatic injury to nerves in the peripheral nervous system (PNS), full functional recovery is poor, despite the well documented ability of axons to regenerate and reinnervate peripheral targets. This poor recovery is an important public health concern. Among the 200,000+ victims of new peripheral nerve injuries in the U.S. each year (72), only ~10% ever recover full function (65). The slowness and inefficiency of axon regeneration is most often blamed for this poor outcome. Regenerating axons from the proximal segment of a cut peripheral nerve must enter a pathway in the distal segment of the nerve and then elongate in that pathway to reach their targets (11). Elongation in the pathway is slow so that if the distance needed to be traversed is long, as they sometimes can be in human patients, a prolonged recovery time will be expected. Elongation of the regenerating axons requires the presence of growth-promoting molecules in the pathway, and over time the ability of supporting cells in the pathway diminishes, which also contributes to poor recovery (34). Enhancing axon regeneration has emerged as a therapeutic target for improving functional recovery after PNS injuries.

Based on earlier hypotheses that neuronal activity might contribute to the process of axon survival and regeneration (e.g., Ref. 40), Gordon and colleagues undertook a systematic evaluation of the effect of electrical stimulation (ES) of cut peripheral nerves on subsequent axon regeneration (3). They discovered that both motor (3) and sensory (29) axon regeneration were enhanced by as little as 1 h of continuous (20 Hz) supramaximal stimulation 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 (3), indicating the activity dependence of the treatment. A number of laboratories have now used ES to enhance axon regeneration in different models of peripheral nerve injury (reviewed in Ref. 76).

On the basis of these results, we showed that moderate daily exercise for 2 wk also enhanced axon regeneration, perhaps exceeding the effects of ES (FIGURE 1) (62). Others have used slightly different exercise protocols to achieve the same end (7, 51, 54, 80, 81), also including swimming (38, 39, 44, 74), and rhythmic limb movements in anesthetized animals (77). Navarro and colleagues even studied the effects of1h of ES followed by daily exercise and found greater enhancement of regeneration than was found with using either treatment alone (7).

**FIGURE 1.** Electrical stimulation and exercise promote axon regeneration in cut peripheral nerves

The common fibular nerves of mice were cut and repaired by end-to-end anastomosis. Lengths of profiles of regenerating axons were measured 2 wk later. Average median axon profile lengths (±SE) are shown for untrained mice, mice that were exercised for 1 h daily during the recovery period, and mice that were exposed to a single hour of electrical stimulation (ES) at the time of nerve repair. Note that both of these therapies result in enhancement of axon regeneration, but exercise is ~50% more potent. Data from Refs. 24, 62.
A common feature to all of these approaches has been assumed to be an increase in the activity of the neurons whose axons are regenerating (76). Increased activity in both sensory neurons and motoneurons is well established with ES. Increased activity of axotomized motoneurons is anticipated during therapies involving walking or swimming, and these activities are driven by the outputs of spinal circuits. Indeed, rhythmic activity in cut ventral roots, presumed to reflect the outputs of the central pattern generators for locomotion, has been described in reduced preparations (e.g., Ref. 52). Activity during treadmill training of motoneurons whose axons have been cut and are regenerating has not been studied specifically. However, it seems unlikely that the outputs of spinal circuitry controlling those behaviors will be suppressed by peripheral nerve transaction, especially because the forelimbs and higher centers that drive lumbar spinal cord pattern generators (37, 66) are left intact and animals are trained quadrupedally. Similarly, activity of sensory neurons whose axons have been cut and are regenerating has not been studied. However, it has been proposed that the central pattern generator for locomotion controls the efficacy of transmission of some primary afferent pathways via primary afferent depolarization (53), but whether these effects might promote increased activity of axotomized sensory neurons during walking/swimming is not known.

The details of the approaches to enhancing axon regeneration using the different experimental therapies described above vary considerably, which complicates any potential translation of these findings to treatments of the wide variety of different peripheral nerve injuries encountered clinically. Approaches involving exercise have the advantage of being low tech, and thus low cost, and they enable patients to participate in and take responsibility for their own recovery. Unfortunately, the large number of variables associated with even the simplest exercise protocol makes the application of exercise to any patient a daunting one. Indeed, even within the experimental studies cited above, differences in the intensity of exercise, the duration of exercise, the pattern of training, the frequency of training sessions, and the time after injury when the exercise was begun are profound.

A number of recent studies have begun to investigate the cellular mechanisms underlying the effectiveness of these activity-associated therapies. In an effort to begin to establish principles for the application of exercise to enhance axon regeneration after injuries to the PNS, in this review we will consider three aspects of the cellular basis for the efficacy of exercise as a therapy that might advance its translational potential: neurotrophins, sex steroid hormones, and neural activity.

**Neurotrophins**

The effectiveness of ES and other activity-associated experimental therapies for treating peripheral nerve injury, such as exercise, requires the ability of the regenerating axons to produce brain-derived neurotrophic factor (BDNF). This neurotrophin has been considered an important promoter of axon elongation during regeneration of peripheral nerves for some time, but in a slightly different cellular context than that invoked to explain the effectiveness of therapies such as ES or exercise. Transformed Schwann cells in the distal segment of cut nerves are known to express BDNF (27, 36), which then acts as a retrograde signal, via trkB receptors on regenerating axons, to stimulate axon regeneration. Treatments of cut and repaired nerves with recombinant BDNF (24, 48) or small molecule trkB agonists (23) promote axon regeneration. Blocking the effect of BDNF with function-blocking antibodies (86) or by using mouse genetics to knock out the BDNF gene selectively in Schwann cells (83) markedly reduces axon outgrowth. Expression of BDNF and its receptor trkB increases transiently in motor and sensory neurons during the first 2–3 days following peripheral nerve transection (33). One hour of ES produces a rapid and marked increase in expression of BDNF and trkB in motoneurons that lasts for at least a week (2). Increased expression of BDNF mRNA in motoneurons is well established following voluntary exercise (32) or treadmill training (59, 83). In mice null for BDNF or trkB specifically in neurons, the effect of exercise is lost completely (23, 83) (FIGURE 2). Expression of the BDNF gene is known to be driven by neuronal activity (41) but also by the downstream effects of activity-stimulated increases in expression of the transcription factor Sox11 (55, 64, 70).

These results all are compatible with the hypothesis first put forth by Gordon and coworkers (4) that therapies such as ES or exercise promote axon regeneration in the PNS by an autocrine or paracrine BDNF signaling mechanism. Secretion of BDNF by growth cones of regenerating axons binds to trkB receptors on the same or adjacent axons to promote growth. Any paracrine stimulation of axon elongation would require BDNF to be secreted from one axon and cross the barriers of at least two endoneurial tubes to stimulate the growth of adjacent axons. Because in mice in which the gene for BDNF is selectively knocked out in only a subset of peripheral axons we found no promotion of regeneration of BDNF-deficient axons from adjacent BDNF-expressing axons (83), we favor an autocrine signaling pathway. This mechanism compliments the traditional...
role of BDNF as a retrograde signal to promote regeneration.

Sensitive assays have now been developed to measure BDNF in serum, and they have been used to show that BDNF protein levels increase in serum with exercise protocols similar to those we have shown to result in enhanced axon regeneration (61). Whether these assays could be applied clinically to screen for the effectiveness of different types of exercise as a therapy for axon regeneration in the PNS is problematic, since the role of BDNF may be so localized to the regenerating axons that its effect might not be detected in serum. However, it would be of considerable interest to know whether measures of serum BDNF concentration would form a reasonable predictor of the success of any exercise protocol in promoting axon regeneration in cut nerves.

An additional impediment to the translation of these findings might be the presence of single nucleotide polymorphisms (SNPs) in the BDNF gene. Several SNPs of this gene have been described, but the best known of these (rs6265) results in a single amino acid (Val66Met) substitution and is found in >25% of the human population (6). The Val66Met substitution is in the prodomain of the BDNF molecule (6), which is cleaved to form mature BDNF either intracellularly, in the trans-Golgi network or secretory vesicles (73), or by plasmin or matrix metalloproteinases after secretion (49, 85). Regulated release of BDNF is impaired in cells containing the Val66Met SNP (12, 14, 22), and in a mouse model of this SNP, the mutant prodomain stimulated growth cone retraction via the common neurtrophin receptor p75NTR (6). Thus individuals with the Val66Met SNP might not be able to respond appropriately to therapies for peripheral nerve injury such as ES or exercise, and their application might even result in an inhibition of axon regeneration. A mouse model of the Val66Met SNP has been developed (13), and its phenotype is remarkably similar to that observed in individuals with this SNP. Evaluating the effectiveness of these experimental therapies for treatment of peripheral nerve injuries in this mouse model should provide important insights as to their efficacy in the human population.

Sex Steroid Hormones

In the course of evaluating the effect of exercise on axon regeneration in mice, we discovered a marked sex difference. In male mice, 1 h of slow treadmill walking daily (continuous training protocol) results in a marked increase in the length of regenerating axons 2 wk later, but the same exercise protocol has no enhancing effect in females. In female mice exposed to daily interval training at a faster treadmill speed, a pattern of activity that is comparable to that observed during voluntary wheel running (20), the enhancement of axon regeneration is impressive, but no enhancement is found in male mice exposed to the same interval training protocol (84).

Sex steroid hormones likely mediate this sex difference. Levels of serum testosterone are significantly increased in continuously exercised male mice but not in continuously exercised females or interval-exercised mice of either sex. Castrating males blocked the enhancing effect of exercise completely and did not influence the ineffectiveness of the interval-training protocol, suggesting a role for gonadally derived androgens. Although no increase in serum testosterone was found in interval-trained females, treatments of unexercised female mice with an inhibitor of P450 aromatase, an enzyme that catalyzes the conversion of testosterone and its precursors into estradiol, resulted in a striking enhancement of the lengths of regenerating axons (84). In subsequent experiments, we found that treating mice with the androgen receptor blocker flutamide blocked the effect of both exercise and ES in both males and females (75) (FIGURE 3).

Based on these findings, we proposed that the effects of therapies such as ES or exercise in pro-
moting axon regeneration in the PNS had an androgenic component in both males and females. Although androgen receptors are found in all motoneurons (26), the identity of cells in which this critical androgen receptor signaling takes place and the relationship between this signaling and ES or exercise on the one hand and increased BDNF/trkB expression on the other are not clear at this time. It is likely there is also an estrogenic component to the enhancement. Estrogen receptors have been shown to increase expression in axotomized lumbar motoneurons (46), and exogenous estrogen administration has been shown to enhance axon regeneration (45, 67, 71) and prevent axotomy-induced cell death (43) following nerve crush. The source of these endogenous sex hormones is not clear; the hormones could be gonadally derived or locally synthesized by neurons or glia. Although the expression of neuronal androgen and estrogen receptors and the effects of gonadal hormones after nervous injuries have been studied extensively, only recently has de novo neuronal steroid synthesis in injury and disease begun to be investigated. Application of findings regarding the role of steroid hormones may be difficult since it is reasonable to assume that neuronal sex steroids will have differential roles intrinsic to the state of the nervous system (developing, aging, injured, sedentary, exercising, male, female, etc.). For example, the androgen receptor blocker flutamide has no effect on normal hippocampal cell proliferation but blocks exercise-induced increases in neurogenesis (60).

Glia in the central nervous system respond to peripheral axotomy and are also known to be responsive to androgens (8, 15, 16, 19, 42) and estrogens (1, 69). Astrocytes express estrogen and androgen receptors (42), and microglia express androgen receptors (28). After brain lesion, reactive astrocytes and microglia may be targets of androgens and estrogens (28). These reactive astrocytes and microglia can also secrete BDNF (57, 68). In addition, microglia express steroid-converting enzymes (35) such as 5 alpha-reductase, which converts testosterone into dihydrotestosterone. The presence of these enzymes suggest that glia may be able to locally produce androgens and could stimulate the release of BDNF in an autocrine and/or paracrine manner. Although microglial-secreted BDNF has been implicated in the maintenance of neuropathic pain after peripheral nerve injury (17, 78) and astrocyte-secreted BDNF increases dramatically after spinal cord injury (21), whether these glia assist or hinder neural regeneration after injury is still not clear.

Expression of BDNF and trkB is stimulated by estrogens (9) and androgens (67, 82) in neurons as well. This hormone-dependent expression appears to have a slower onset but a longer time course of expression than found after ES (67). If exercise is enhancing axon regeneration by both promoting neuronal activity similar to ES and stimulating the release of sex steroids, then exercise may result in both a rapid onset and a prolonged duration of increased expression of BDNF and trkB in axotomized neurons and/or surrounding reactive glia, but this has not been studied. Determining the time dependence of these mechanisms will be important to create appropriate exercise-based therapies for patients.

Although it is clear that neuronal BDNF as well as androgen receptor signaling is necessary for axon regeneration, the details of how exercise increases androgens is less clear. Axotomized neurons likely synthesize androgens de novo or are supplied androgens or androgenic precursors by neighboring glia. We hypothesize that exercise- or ES-induced neuronal activity increases local neuronal androgen levels by stimulating expression of the enzymes (17β-

**FIGURE 3. Androgens are required for enhancement of regeneration by exercise or ES**

A: average (+95% confidence limits) fold changes in median lengths of profiles of regenerating axons 2 wk after the common fibular nerve was cut and repaired are shown for male and female mice. Animals were exercised using either slow continuous walking for 1 h or a more intense interval training strategy. Some animals were treated with a sustained release dosage form of the androgen receptor blocker flutamide, with either sex-appropriate exercise or no exercise. Animals in one group of male mice were castrated at the time of nerve repair. Data from Refs. 72, 81. B: similar flutamide treatment blocked the enhancing effect of a single application of ES in both male and female mice. Data are from Ref. 75.
Increased neuronal activity alone is sufficient to
result in the regeneration of motor axons expressing ChR2 and not axons that do not contain the transgene (FIGURE 4) (47). These results are consistent with the hypothesis that in-vitro studies by Berchtold and colleagues (9) found that ovariotomizing female rats immediately reduced voluntary exercise levels and dramatically reduced BDNF mRNA levels in the hippocampus. Restoring estrogen to intact control levels immediately restored voluntary exercise levels and slowly returned BDNF mRNA expression to control levels (9). This interaction of estrogen and BDNF could be due to genomic regulation of the BDNF gene via the activation of the estrogen response element on the BDNF gene or various nongenomic mechanisms (56).

Discovering how expression of these two pathways is affected by injury to the PNS and neuronal activity in male and female animals may reveal new pharmacological targets and will aid in the translation of experimental activity-associated therapies. The two hormone pathways may be acting independently or synergistically to promote axon regeneration (67, 71). Because androgen (and likely estrogen) receptor signaling is required in both males and females for the effectiveness of therapies such as ES or exercise, their application in patients with steroid hormone deficiency, a history of prostate cancer, or postmenopausal women should be investigated more thoroughly.

Neuronal Activity

The consensus reached in the earliest studies of the effects of exercise in enhancing recovery from spinal cord injury was that the effects of exercise were the result of increased neuronal activity. In more recent papers, other explanations have been put forth, including environmental enrichment (31), increased caloric utilization (30), and intermittent hypoxia (18). Using mice in which the light-sensitive cation channel channelrhodopsin (ChR2) is expressed in some but not all axons in peripheral nerves, we have been able to show that increasing neuronal activity using light results in a selective enhancement of regeneration of motor axons expressing ChR2 and not axons that do not contain the transgene (FIGURE 4) (47). These results are consistent with the hypothesis that increased neuronal activity alone is sufficient to enhance axon regeneration. However, a number of important questions remain unanswered. The nature of the increase in neural activity, the minimal amount of increase that is sufficient, and whether sensory and motor axons have the same requirements for increased activity are not now known but are amenable to future study using this optogenetics approach. Indeed, although it is clear that exercise leads to an enhancement of regeneration of motor axons (see below), its effect on the regeneration of sensory axons is relatively unknown. The use of retrograde labeling to study sensory axon regeneration after different forms of exercise should be a future priority.

In addition to any requirements for increased activity, establishing the appropriate timing of that increase will be important. In rats that were treadmill trained daily for 2 wk beginning immediately (3 days) after sciatic nerve transection and repair, we studied the restoration of the compound muscle action potential (M response) recorded from reinnervated muscles and that of the H reflex, the muscle potential evoked in response to sensory axon stimulation (10). These results were compared with a series of rats in which the same 2-wk exercise protocol was applied but delayed until the first sign of muscle reinnervation, −3 wk after injury, and to a group of untrained controls. By 8−10 wk after injury, the amplitudes of the restored M responses in both the immediately trained rats and those whose training was delayed were significantly greater than those found in untrained controls (FIGURE 5A). Using a slightly different immediate treatment (untreated; n = 4), cut axons were activated by blue light for 1 h before nerve repair. In control mice, nerves were cut and repaired with no treatment (untreated; n = 8), 1 h of ES (n = 5), or 2 wk of daily treadmill exercise (n = 7). Means ± SE fold changes in lengths of re-generating axons relative to untreated mice are shown. The horizontal dashed line at 1.0 reflects the amount of regeneration observed in untreated animals. Mean fold changes for all three groups shown are significantly greater than for untrained mice.

Data are from Refs. 47, 62.
training protocol, Navarro and colleagues (7) also found an enhancement of motor axon regeneration leading to a larger M response. In contrast, the amplitude of the restored H reflexes differed in the different groups. In untreated control rats, a transient increase in the amplitude of the restored H reflex is followed by a significant decrease, relative to that found in intact rats (10, 58). In immediately trained rats, the exaggerated reflex response is retained for at least 8–10 wk (FIGURE 5B) (10). If the application of exercise is delayed, the size of the restored H reflex at this recovery time is the same as that found in untreated rats (FIGURE 5B).

Whether these different effects of the timing of initiation of exercise on the plasticity of spinal circuits following injury to the PNS are the result of differences in the enhancing effects of exercise on sensory vs. motor axon regeneration or whether they are influenced by the rearrangements of synaptic inputs onto motoneurons that accompany peripheral axotomy (e.g., Ref. 5) is not clear at this time. A marked difference in the outcome of the effect of exercise on synaptic plasticity may depend on when that treatment is applied. More studies are needed to define the therapeutic window for application of exercise.

We also have investigated the effects of changing the intensity or amplitude of the applied exercise. Rats and mice were exercised for 1 h daily, 5 days/wk, for 2 wk by walking slowly on an upward-inclined treadmill. Training was begun on day 3 after sciatic nerve transection and repair. This immediate upslope training was expected to result in the recruitment of more motor units into activity than level training, since the amplitude of EMG activity in both flexor and extensor muscles is increased with slope (63). Since during upslope walking muscles undergo very little lengthening (50), we expected upslope training to result in decreased activity in sensory axons encoding stretch of intact synergist muscles that feed back onto the axotomized motoneurons.

In mice, we found that upslope training resulted in the successful regeneration of significantly more motor axons during the first 2 post-transection weeks than either untrained or level-trained animals (25). More sciatic motoneurons could be retrogradely labeled from tracer application 4 mm distal to the injury site in upslope-trained mice (240.98 ± 9.64, mean ± SE) than in untrained animals (38.20 ± 6.76), level-trained animals (151.09 ± 21.51), or animals treated with 1 h of ES (109.33 ± 27.22). Recruiting more motoneurons into activity during exercise produced the anticipated enhancement of motor axon regeneration.

In rats, we studied the amplitudes of the restored M responses and H reflexes in groups of animals exposed to 2 wk of upslope training, level training, or no training. The amplitudes of the restored M responses 8–10 wk after injury was significantly greater in both level- and upslope-trained rats than in untrained controls (FIGURE 5A), but no significant differences were found between level- and upslope-training groups. Whether the mouse-rat difference in the effect of upslope training was the result of differences in the types of outcome measures used in the two species or whether it reflects significant changes in motor unit reinnervation following the cessation of the exercise is not clear at this time. In contrast, the effect of upslope training on the amplitude of the restored H reflex is striking. Unlike the effect of level training, where an exaggerated H reflex is found 8–10 wk after nerve repair, a significant decrease in H reflex amplitude was found in upslope-trained rats relative to both level-trained and untrained animals. Whether this markedly different outcome was obtained simply

![FIGURE 5. Outcomes of exercise on axon regeneration and functional recovery may differ for different kinds of axons and the treatment applied](http://physiologyonline.physiology.org/)

A: in rats treated with 2 wk of daily moderate treadmill training, the amplitude of the direct muscle (M) response to stimulation of regenerated axons is increased markedly 8–10 wk after injury, whether the exercise was begun immediately (3 days after transection), delayed for 3 wk, or conducted using an upslope inclined treadmill. Mean amplitudes (±SE; n = 6 for each group) of M responses, scaled to the pre-transection M response amplitude (horizontal dashed line) are shown. B: similar data for the amplitude of the H reflex in these three groups. In animals of the immediate group, H-reflex amplitude is increased by exercise. In animals in the upslope group, the training actually decreases the amplitude of the H reflex. Some data are from Ref. 62, 63.
by changing the exercise conditions reflects an effect on the sensory axon regeneration, the efficacy of the synaptic inputs onto the injured motoneurons, or both awaits further study. The nature of the exercise protocol applied can change the nature of at least this simple spinal circuit. It will be of considerable interest to study the effects of downslope treadmill training on restoration of muscle innervation and this spinal circuitry. Recruitment of fewer motoneurons into activity would be expected to occur during downslope walking than level walking (63), but activity of stretch-sensitive afferent neurons in intact synergists might be expected to be greater during downslope walking where prolonged periods of muscle stretch are encountered (50). Evaluation of the effects of different exercise protocols on postinjury properties of other reflex pathways, such as the crossed extensor reflex (79), will be equally interesting.

Conclusions

Activity-associated therapies such as exercise could be applied widely to enhance functional recovery following peripheral nerve injuries, addressing an important public health issue. They are inexpensive and enable patients to assume responsibility for their own recovery. However, application of these therapies to diverse types of nerve injuries in a diverse human population will require exercise prescriptions that are unique to the injured nerve, the desired regeneration of different types of axons, and the developmental stage and genetic background of the patients. Some principles that might form a basis for developing such prescriptions are emerging from a greater understanding of the cellular mechanisms underlying these therapies, but more studies are needed.

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