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Exercise promotes recovery after motoneuron injury via hormonal mechanisms

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Abstract

Injuries to spinal motoneurons manifest in a variety of forms, including damage to peripheral axons, neurodegenerative disease, or direct insult centrally. Such injuries produce a variety of negative structural and functional changes in both the directly affected and neighboring motoneurons. Exercise is a relatively simple behavioral intervention that has been demonstrated to protect against, and accelerate recovery from, these negative changes. In this article, we describe how exercise is neuroprotective for motoneurons, accelerating axon regeneration following axotomy and attenuating dendritic atrophy following the death of neighboring motoneurons. In both of these injury models, the positive effects of exercise have been found to be dependent on gonadal hormone action. Here we describe a model in which exercise, hormones, and brain-derived neurotrophic factor might all interact to produce neuroprotective effects on motoneuron structure following neural injury.

Key Words: axon regeneration; axotomy; dendritic morphology; exercise; hormones; neuroprotection; neurotrophins

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Introduction

Neurodegenerative diseases and neural injuries can result in the loss of spinal motoneurons, potentially leading to compromised quality of life or more severe pathologies. For example, motor neuron diseases (e.g., amyotrophic lateral sclerosis; Cleveland and Rothstein, 2001) or spinal cord injury result in the loss of spinal motoneurons and motor dysfunction. Importantly, surviving motoneurons also show a variety of morphological and functional changes after such insults. For example, motoneurons undergo dendritic atrophy after spinal cord injury (Sengelaub and Xu, 2018), and following proximal motor root lesions, surviving motoneurons form dendraxons and have reduced dendritic morphology and function (Lindå et al., 1992; Bergerot et al., 2004). Although most motoneurons survive more distal peripheral nerve injuries (e.g., axotomy) in adult mammals, such injuries result in the immediate loss of voluntary motor control and rapid degeneration of the distal axonal segments. Centrally, axotomy results in motoneuron somal and dendritic atrophy, as well as a withdrawal of synaptic inputs from somata and proximal dendrites.

Developing therapeutic strategies to protect surviving motoneurons and/or accelerate recovery of function is an important goal. Exercise has been strongly supported by the scientific community as beneficial for maintaining cognitive ability, emotional well-being, and overall good physical health. In this review, we will briefly review the effects of exercise on promoting recovery of both the peripheral (axons) and central (dendrites) components of spinal motoneurons after injury, highlighting the role of androgens in mediating these therapeutic effects. Testosterone plays a well-documented role in driving adaptive changes following exercise, with the classical dogma stating that exercise increases testosterone output in order to build muscle mass as a means of repairing damage incurred during the exercise session (Bhasin et al., 1996). As we discuss below, the upregulation of endogenous testosterone following exercise may also be a mechanism that contributes to the pro-regenerative and neuroprotective phenotypes seen with exercise following neural injury.

Search Strategy and Selection Criteria

Database used to indentify the most relevant papers included in this article: https://www.ncbi.nlm.nih.gov/pubmed/. Keywords for searching (selection criteria): axon regeneration, axotomy, dendritic morphology, exercise, testosterone, BDNF, neuroprotection. Dates of searching: 1990–2019.

Testosterone, Exercise, and Regeneration after Axotomy

Following axotomy, the severed distal axonal segments undergo obligatory Wallerian degeneration within days of injury (Brushart, 2011). Recovery of function occurs by natural regeneration that slowly, partially, and non-specifically reinnervates denervated distal target tissues. Furthermore, denervated target tissues may irreversibly atrophy before re-innervation occurs, and recovery is often poor (Brushart, 2011). Distal target tissues are particularly vulnerable following denervation, as re-innervation by axonal regeneration requires longer times, during which the denervated tissues continue to atrophy.

Treatment with testosterone accelerates both axon regeneration and functional recovery following axotomy of spinal or cranial motoneurons (Foecking et al., 2015). This

effect is androgen receptor-dependent, and blocking the androgen receptor prevents testosterone-induced enhanced regeneration (Foecking et al., 2015). Exercise has also been demonstrated to be neurotherapeutic after axotomy, increasing both the number of regenerating axons sprouting from the proximal stump of the axotomized fibular nerve and axon elongation in the injured peripheral nerve when compared to non-exercised animals (Sabatier et al., 2008). Androgens have been directly implicated in the positive effects of exercise after axonal injury. For example, treadmill training results in both increases in serum testosterone levels and enhances axon regeneration after axotomy in male rats; castration via surgical removal of the testes (orchidectomy) prevents treadmill training effects on axon regeneration (Wood et al., 2012). In the same axotomy model, treatment of exercised animals with the androgen receptor antagonist flutamide reduces the median length of regenerating axons to lengths comparable with animals who did not exercise (Thompson et al., 2014) suggesting that androgen receptor action is a necessary driver of the neuroprotective benefits of exercise following injury.

Exercise has been demonstrated to enhance axon regeneration in both males and females (Wood et al., 2012; Thompson et al., 2014). However, males and females require different exercise protocols to enhance axon regeneration. Males require continuous treadmill training, while females require high-intensity interval treadmill training (Wood et al., 2012). Furthermore, males also show an elevation of their serum testosterone concentrations, while females do not (Wood et al., 2012). Interestingly, systemic blockade of androgen receptors prevents exercise enhancement of axon regeneration in both sexes (Thompson et al., 2014). Together, these findings suggest that exercise-mediated axonal regeneration depends on androgen receptor action, irrespective of sex, exercise modality, and effects on prolonged elevation of serum testosterone.

Testosterone, Exercise, and Neuroprotection of Motoneuron Dendrites

Research in our lab has examined the effects of motoneuron loss on the structure and function of surviving motoneurons. Our previous studies have demonstrated that surviving motoneurons respond to the loss of their neighbors with marked dendritic atrophy that results in reduced excitability of the remaining motoneurons (Little et al., 2009; **Figures 1** and **2**).

Treatment with physiological doses of testosterone attenuates dendritic atrophy induced by motoneuron loss (see **Figures 1** and **2** for details), as well as the attenuated excitability, in surviving motoneurons (Little et al., 2009). Furthermore, we have also found that this neuroprotective effect of testosterone is dependent on androgen receptor action (Cai et al., 2017). Similar to the effects of exercise on axon regeneration discussed above, we recently demonstrated that exercise attenuates induced dendritic atrophy in surviving motoneurons following the death of nearby motoneurons (**Figures 1** and **2**; Chew and Sengelaub, 2019). This attenuation of dendritic

atrophy with exercise was of a comparable magnitude to that seen with exogenous testosterone treatment. Androgens have also been directly implicated in the positive effects of exercise on protecting motoneurons from dendritic atrophy. Following the loss of endogenous testosterone after castration, male rats allowed to exercise did not show the attenuation of dendritic atrophy seen in exercised gonadally intact rats (**Figures 1** and **2**; Chew and Sengelaub, unpublished). Again, this suggests that androgen action is a necessary driver of the neuroprotective benefits of exercise following injury.

Testosterone, Exercise, and Neuroprotection with Neurotrophic Factors

This dependence on the efficacy of exercise on testosterone and the anabolic action of testosterone in adaptive effects following exercise suggest that the neuroprotective effects of exercise may utilize the same androgen receptor-driven mechanism seen with exogenous testosterone treatment. One possible mechanism for these exercise effects could be via interactions with brain-derived neurotrophic factor (BDNF). BDNF is a neurotrophin that has been implicated in promoting neuroplasticity in both the brain and spinal cord. For example, application of recombinant BDNF to cut nerves enhances axon regeneration (McGregor and English, 2019). Similarly, injection of bone marrow stromal cells and lentiviral vectors expressing BDNF significantly enhances axonal regeneration after nerve segment ablation (Gao et al., 2016). Exercise effects on axon regeneration and the maintenance of synaptic terminals on motoneurons requires BDNF-trkB signaling (McGregor and English, 2019). These BDNF-mediated effects could be part a common mechanism that has been hypothesized for the effects of exercise and androgens on motoneuron dendrites following partial motoneuron depletion (Chew and Sengelaub, 2019) and axonal regeneration following axotomy (Thompson et al., 2014). It is thought that exercise induces increases in testosterone that in turn upregulates BDNF and its receptor, trkB (McGregor and English, 2019).

Similarly, neurotrophic signaling by BDNF has been demonstrated to exert a regulatory effect on motoneuron dendritic morphology (Verhovshek et al., 2013). For example, axotomy results in dendritic retraction in motoneurons, and application of BDNF to cut nerves restores dendritic length. However, this protective effect of BDNF is only observed when testosterone is present, and treatment with either BDNF or testosterone alone is ineffective (Verhovshek et al., 2013). It has been demonstrated that androgens regulate BDNF levels and trkB receptors in motoneurons (Verhovshek et al., 2013). Thus, as with axotomy, the increase in androgen production following exercise could upregulate neurotrophic signaling, resulting in neuroprotection from induced dendritic atrophy in surviving motoneurons following the death of nearby motoneurons.

Potential Alternative Mechanisms

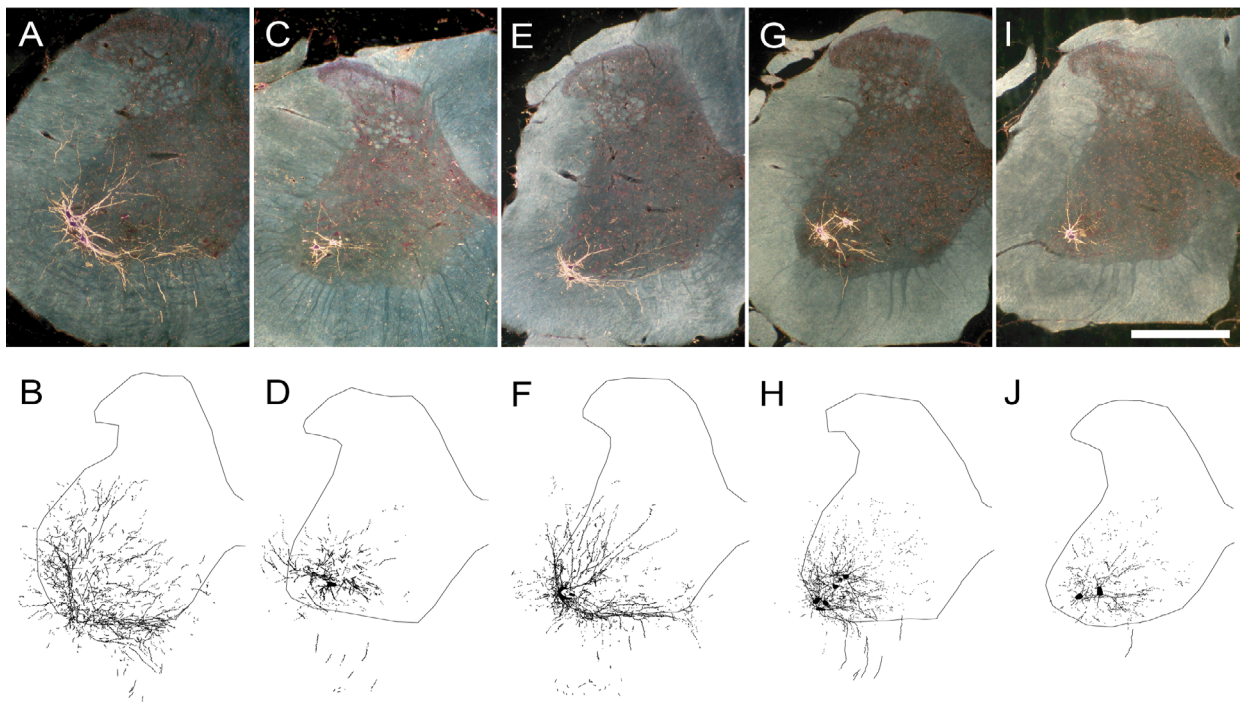Direct mediation of neuroplasticity is not the only possible

Figure 1 Motoneuron morphology is protected by gonadal hormones or exercise following the death of neighboring motoneurons.
Darkfield digital micrographs of transverse hemisections through the lumbar (L2) spinal cord and computer-generated reconstructions of cholera toxin-conjugated horseradish peroxidase (BHRP)-labeled somata and processes of an untreated male (A, B), and saporin-injected male rats with either no further treatment (C, D), or given exogenous testosterone (E, F), *ad libitum* exercise (G, H), or *ad libitum* exercise after orchidectomy (I, J) after BHRP injection into the left vastus lateralis muscle. Treatment with testosterone (E, F) was achieved via Silastic capsule (45 mm long; outer diameter = 3.18 mm; inner diameter = 1.57 mm) implanted subcutaneously (from Little et al., 2009); such implants produce plasma titers of testosterone in the normal physiological range (Smith et al., 1977). Exercised animals (G, H, I, J) had running wheels attached to their home cages, and exercise was *ad libitum* (from Chew and Sengelaub, 2019 and unpublished data). The treatment periods with testosterone or exercise after saporin injection was four weeks. Computer-generated composites of BHRP labeling were drawn at 480 μm intervals through the entire rostrocaudal extent of the quadriceps motor pool; these composites were selected because they are representative of their respective group average dendritic lengths. Scale bar: 500 μm.
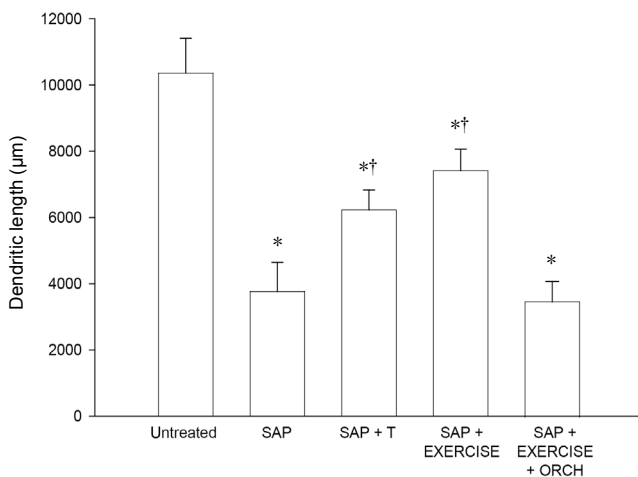

Figure 2 Motoneuron dendritic length is protected by gonadal hormones and exercise following the death of neighboring motoneurons.
Dendritic lengths of quadriceps motoneurons in untreated animals ($n = 5$), saporin-injected animals that either received no further treatment (SAP; $n = 6$) or were given exogenous testosterone (SAP + T; $n = 6$), *ad libitum* exercise (SAP + EXERCISE; $n = 6$), or *ad libitum* exercise after orchidectomy (SAP + EXERCISE + ORCH; $n = 5$). Following saporin-induced motoneuron death, surviving neighboring motoneurons lost almost 64% of their dendritic length. Treatment with physiological testosterone via Silastic capsule (45 mm long; outer diameter = 3.18 mm; inner diameter = 1.57 mm) implanted subcutaneously (Little et al., 2009), or *ad libitum* running wheel exercise, attenuated this dendritic atrophy (Chew and Sengelaub, 2019); neuroprotective exercise effects were prevented with orchidectomy (Chew and Sengelaub, unpublished). Tissue was collected 4 weeks after SAP injection. Dendritic lengths were reconstructed in three dimensions under darkfield illumination at final magnification of 250× using Neurolucida, and total dendritic length was corrected by the number of BHRP-labeled motoneurons in each animal. Groups were compared using analysis of variance. Bar heights represent mean ± SEM. *indicates significantly different from untreated animals. †indicates significantly different from untreated saporin-injected animals. BHRP: Cholera toxin-conjugated horseradish peroxidase.

mechanism by which exercise may be neuroprotective. Exercise upregulates chemical signals that promote vascularization [e.g., vascular endothelial growth factor (VEGF)]. As VEGF promotes new vasculature formation, biochemical resources are more effectively able to be transported to the tissues undergoing reparative/restorative processes. Thus, exercise may upregulate VEGF and increased vascularization, which promotes neuroprotection/regeneration.

Exercise also increases production of antioxidant species and heat-shock proteins as a likely response to the increased production of oxidizing byproducts as a result of the increased metabolic and stress demands placed on the body during exercise. Some of these adaptations can persist beyond the cessation of a single exercise session and may be long lasting (Mattson, 2012), and it stands to reason that maintenance of a regular exercise regimen can maintain these beneficial adaptations. Greater capacity to safely metabolize reactive oxygen species and protect endogenous protein function may stabilize a disrupted extracellular environment seen following injury.

Endurance exercise, in particular, also produce mitochondrial changes in non-neuronal cells and neurons that may contribute to neuroprotection and/or regeneration. For example, endurance exercise causes muscle cells to increase their oxidative metabolic capacity by increasing the number of mitochondria and oxidative enzymes present in the cells (Mattson, 2012). Newly developing hippocampal neurons in adult mice show robust increases in mitochondrial number and volume of individual mitochondria that coincide with dendritic outgrowth (Steib et al., 2014). These increases in total mitochondrial volume were also found to be heavily driven by mitochondria located within the dendritic arbor, suggesting a relationship between neurite growth and increased energetic demands. Thus, endurance exercise may accelerate early mitochondrial proliferation and dendritic outgrowth, providing evidence that increased number of mitochondria can provide the necessary metabolic machinery to fuel more rapid neurite extension.

Interestingly, many of the benefits of exercise outlined here have also been linked to BDNF and/or androgen signaling mechanisms. In addition to the effects on axons and dendrites described above, testosterone has also been shown to increase production of VEGF (Chodari et al., 2016) and heat-shock proteins (Ahlbom et al., 2001), and BDNF has also been implicated in increases in VEGF production and angiogenesis (Mattson, 2012). While we cannot say that any specific adaptation or molecular pathway is primarily responsible for the neuroprotective or therapeutic effects of exercise, it seems likely that exercise may cause multiple physiological adaptions that share conserved signaling pathways to create an environment that promotes neuroprotection or more effective recovery following injury.

Conclusions and Future Avenues

Exercise offers a simple, low barrier-to-entry behavioral intervention that has been demonstrated to be an effective neuroprotective and pro-regenerative following neural injury. Most of the benefits of exercise discussed here have focused on examining how exercise is beneficial after injury has been sustained, but many of the non-neural benefits of exercise occur as reductions of health risk factors. It is near consensus opinion within the medical field that regular exercise helps lower risk of cardiovascular disease, stroke, diabetes, and age-related cognitive decline, but there has been relatively little research into how prior exercise may affect recover from subsequent neural injuries. In this review, we have outlined how androgens and exercise may drive neuroprotective effects. What remains to be seen is whether exercise can cause long-term adaptations that can reduce the risk of or severity to neural injuries, in a similar fashion to the risk reductions seen in cardiovascular disease.

Future avenues of research could examine whether and how exercise modulates or causes adaptations to the androgen sensitivity or metabolic properties of skeletal muscle, and whether any adaptations have upstream effects on innervating motoneurons. Such adaptations could potentially offer insight to how mechanisms that contribute to hypertrophy and strengthening of muscles with exercise may reduce initial susceptibility of their motoneurons to disease/injury, how severely they are affected by such insults, and ultimately their responsiveness to treatment and potential to return to a normal state.

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