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BDNF AND ANDROGEN INTERACTIONS IN SPINAL NEUROMUSCULAR SYSTEMS

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Abstract

Neurotrophic factors and steroid hormones interact to regulate a variety of neuronal processes such as neurite outgrowth, differentiation, and neuroprotection. The coexpression of steroid hormone and neurotrophin receptor mRNAs and proteins, as well as their reciprocal regulation provides the necessary substrates for such interactions to occur. This review will focus on androgen-BDNF interactions in the spinal cord, describing androgen regulation of BDNF in neuromuscular systems following castration, androgen manipulation, and injury. Androgens interact with BDNF during development to regulate normally-occurring motoneuron death, and in adulthood, androgen-BDNF interactions are involved in the maintenance of several features of neuromuscular systems. Androgens regulate BDNF and trkB expression in spinal motoneurons. Androgens also regulate BDNF levels in the target musculature, and androgenic action at the muscle regulates BDNF levels in motoneurons. These interactions have important implications for the maintenance of motoneuron morphology. Finally, androgens interact with BDNF after injury, influencing soma size, dendritic morphology, and axon regeneration. Together, these findings provide further insight into the development and maintenance of neuromuscular systems and have implications for the neurotherapeutic/neuroprotective roles of androgens and trophic factors in the treatment of motoneuron disease and recovery from injury.

Keywords

testosterone; motoneuron; muscle; morphology; neurotrophic factors

Introduction

Neurotrophic factors and steroid hormones interact to regulate a variety of neuronal processes such as neurite outgrowth, differentiation and neuroprotection. The coexpression of steroid hormone and neurotrophin receptor mRNAs and proteins (Toran-Allerand et al., 1992; Toran-Allerand, 1996; Shugrue et al., 2000), as well as their reciprocal regulation

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(Sohrabji et al., 1994) provides the necessary substrates for such interactions to occur. Steroid hormone receptors and neurotrophin receptors and their ligands are coexpressed in several brain regions, including the hippocampus, cerebral cortex, cingulate cortex, olfactory bulb and basal ganglia (Miranda et al., 1993; Jezierski and Sohrabji, 2000). For example, estrogens support brain-derived neurotrophic factor (BDNF) mRNA and protein expression in hippocampal, basal forebrain, and pyriform cortical neurons (Gibbs, 1999; Jezierski and Sohrabji, 2001; Solum and Handa, 2002). In addition, cross-coupling of steroid hormone and neurotrophic factor intracellular signaling pathways underlies the activation of genes involved in neurite outgrowth and differentiation (Toran-Allerand et al., 1999). Furthermore, the gene encoding BDNF contains an estrogen response element (Sohrabji et al., 1995), which provides a direct mechanism for steroid hormone control of BDNF expression.

These steroid hormone-neurotrophic factor interactions not only occur in the brain but throughout the central and peripheral nervous system. This review will focus on androgen-BDNF interactions in the spinal cord, and in particular, on androgenic regulation of BDNF in neuromuscular systems following androgen manipulation, and injury. The effects of androgen and BDNF treatment on motoneuron morphology and BDNF, trkB, and androgen receptor expression, in sexually dimorphic and somatic motoneuron populations and their target muscles will be discussed.

BDNF is a member of the neurotrophin family of neurotrophic factors and binds with high affinity to the trkB receptor. BDNF is expressed throughout the body, including in the central (Yan et al., 1997) and peripheral nervous system (Matsuoka et al., 1991; Griesbeck et al., 1995) and skeletal muscles (Kust et al., 2002; Mousavi et al., 2004; Verhovshek et al., 2010). BDNF is produced in limited quantities in target tissues and retrogradely transported back to the CNS where it exerts a variety of effects (Thoenen, 1991). Indeed, BDNF is retrogradely transported from skeletal muscle to motoneurons (DiStefano et al., 1992; Koliatsos et al., 1993). Thus, levels of BDNF protein in spinal motoneuron somata can be altered through either changes in peripheral production or retrograde transport. However, BDNF is also produced centrally and released in an autocrine and/or paracrine manner within neuronal populations (Lom and Cohen-Cory, 1999; Lom et al., 2002; Horch, 2004), including spinal motoneurons (Buck et al., 2000). Thus, levels of BDNF protein in spinal motoneuron somata could also be altered through changes in BDNF message in the motoneurons themselves, local production of BDNF, or even differential trafficking into dendrites. The actions of BDNF in the central nervous system have traditionally been thought of as facilitative, promoting growth or survival of neurons (Snider, 1994; McAllister et al., 1999; Pitts et al., 2006). For example, BDNF supports neuron survival (Henderson et al., 1993; Rasika et al., 1999; Xu et al., 2001), somal area (Wissman and Brenowitz, 2009), axonal outgrowth (Cabelli et al., 1995; Cohen-Cory and Fraser, 1995; Lom and Cohen-Cory, 1999; Mamounas et al., 2000), and dendritic branching *in vitro* and *in vivo* (McAllister et al., 1995; McAllister et al., 1997; Horch and Katz, 2002; Finsterwald et al., 2009).

BDNF-androgen interactions: maintenance of structure

Sexually dimorphic neuromuscular systems

The lumbar spinal cord of male rats contains a sexually dimorphic motor nucleus, the spinal nucleus of the bulbocavernosus (SNB; Breedlove and Arnold, 1980) that consists of approximately 200 medially-located motoneurons (Arnold et al., 1988). SNB motoneurons innervate the bulbocavernosus and levator ani muscles of the perineum (McKenna and Nadelhaft, 1986), and control penile reflexes important for copulatory behavior (Sachs, 1982). Androgenic effects on SNB motoneurons were first identified over 30 years ago and since then almost every feature of the SNB neuromuscular system has been shown to be regulated by gonadal hormones in some way (Sengelaub and Forger, 2008). The SNB target

musculature plays a critical role in mediating androgenic effects in the SNB neuromuscular system. Maintenance of SNB soma size is regulated by androgens and influenced by motoneuron contact with the SNB target muscles (Araki et al., 1991). Removal of the target musculature in early postnatal life downregulates androgen receptor expression, blocking androgen sensitivity in SNB motoneurons (Lubischer and Arnold, 1995). Furthermore, androgens are thought to control normally-occurring cell death in the SNB (Fishman and Breedlove, 1992) and regulate SNB dendritic morphology by acting at the SNB target musculature (Rand and Breedlove, 1995). It had been suggested that this peripheral regulation of SNB motoneuron morphology results from the actions of target-derived trophic substances (Breedlove, 1986) such as BDNF, and more recent work supports this hypothesis (see below).

Motoneurons

SNB motoneurons express BDNF mRNA and protein, and this expression is regulated by androgens (Ottem et al., 2007; Verhovshek et al., 2010). Castration of adult male rats reduced BDNF mRNA and protein in SNB somata and treating castrates with testosterone maintained BDNF message and protein at levels similar to those of gonadally intact males (Ottem et al., 2007). Interestingly, due to the unusual structure of the BDNF gene, BDNF mRNA can be regulated in various ways. The *Bdnf* gene contains multiple promoters that control transcription of a number of 5' non-coding exons and a common 3' exon coding for the BDNF protein. Through the use of multiple promoters and alternative splicing, a variety of BDNF transcripts are produced from the *Bdnf* gene, all coding for the BDNF protein, but containing different 5' exons (Timmusk et al., 1993). To add to the diversity of BDNF transcripts produced, the *Bdnf* gene contains multiple polyadenylation sites that produce BDNF mRNA with short or long 3' UTRs (An et al., 2008). The diversity of BDNF mRNA produced from a single gene allows for tissue-specific expression of BDNF mRNA and protein (An et al., 2008; Timmusk et al., 1993).

The androgenic regulation of BDNF mRNA is isoform-specific: 5' non-coding exon VI was the only identified isoform that displayed androgen regulation, and its expression decreased after castration, but was restored to levels of those found in gonadally intact males in castrates treated with testosterone (Ottem et al., 2010). This exon-specific androgenic regulation of BDNF mRNA provides a mechanism for localization of BDNF within SNB motoneurons in response to androgen manipulation, as transcript-specific localization of BDNF mRNA to somata and dendrites has been previously demonstrated (An et al., 2008; Ottem et al., 2010; Pattabiraman et al., 2005). Additionally, BDNF protein is reduced in SNB motoneurons (Verhovshek et al., 2010) and proximal dendrites (Ottem et al., 2007) following castration, and testosterone treatment prevented these castration-induced decreases in SNB motoneurons. Together, these findings demonstrate that androgen depletion results in a concomitant decrease in BDNF that is restored by testosterone treatment. Further, this alteration of BDNF protein may reflect an androgenic regulation of BDNF mRNA expression.

Similarly, trkB, the high-affinity BDNF receptor, is present in SNB motoneurons, and its expression is sensitive to androgens (Osborne et al., 2007; Ottem et al., 2007). In adult male rats, castration decreased trkB mRNA (Ottem et al., 2007) and protein (Osborne et al., 2007) in SNB motoneurons and testosterone treatment can prevent or restore this castration-induced trkB downregulation (Osborne et al., 2007; Ottem et al., 2007).

Although the castration-induced decreases in BDNF mRNA and protein in SNB motoneurons demonstrate that changes in systemic androgen levels regulate neurotrophin levels in the SNB, it does not elucidate the site of action for this androgenic regulation of BDNF in these motoneurons. Because SNB motoneurons express androgen receptors

(Matsumoto et al., 1996) it is possible that androgens act directly at the motoneuron to regulate BDNF production. However, the SNB target muscles also express androgen receptors (Monks et al., 2004; Monks et al., 2006), implicating the muscle as a potential site for androgenic regulation of BDNF in SNB motoneurons. Indeed, the dendritic morphology of SNB motoneurons can be regulated by androgenic action at the SNB target musculature (Rand and Breedlove, 1995), demonstrating that the peripheral action of androgens can regulate motoneuron morphology. Furthermore, BDNF is expressed in the SNB target musculature (Verhovshek et al., 2010), can be retrogradely transported from skeletal muscle to spinal motoneurons (DiStefano et al., 1992; Koliatsos et al., 1993), and its role as a retrogradely transported, target-derived neurotrophic factor has been well established (Huang and Reichart, 2001), providing a likely mechanism for the peripheral androgenic regulation of BDNF levels in SNB motoneurons. Recent evidence suggests that BDNF can act in an autocrine fashion to increase local BDNF levels (Cheng et al., 2011), allowing for local changes in the amount of BDNF available for retrograde transport from the target muscle to motoneurons. Based on these data, it is possible that androgens act at the SNB target muscle to locally increase levels of BDNF, and excess BDNF could be retrogradely transported, resulting in an increase in BDNF protein in SNB motoneurons following androgen treatment in castrated male rats. In fact, restricting testosterone treatment in castrated males directly to the SNB target musculature via a microimplant affixed directly to the SNB target musculature was sufficient to maintain the staining intensity of BDNF-immunolabeled SNB somata at those of normal males (Verhovshek and Sengelaub, 2010a; Fig. 1). Placement of the same implant in the interscapular region had no effect on BDNF immunolabeling, demonstrating that a site of action for androgenic regulation of BDNF in the SNB is the target musculature. Furthermore, in gonadally intact adult male rats treated with microimplants at the target musculature containing the androgen receptor blocker hydroxyflutamide, the staining intensity of BDNF-immunolabeled SNB somata was decreased compared to gonadally intact animals that had the same microimplants placed interscapularly or when compared to castrated males with a testosterone microimplant at the target muscle. These results demonstrate that the SNB target musculature is a critical site of action for the androgenic regulation of BDNF protein in SNB motoneurons and that this androgenic regulation of BDNF occurs through the peripheral action of androgens via androgen receptors.

As previously mentioned, changes in the levels of BDNF in SNB motoneurons could occur through a variety of mechanisms. Treatment of castrates with androgen at the target muscle could increase the amount of peripherally-produced and/or retrogradely transported BDNF, resulting in maintenance of BDNF immunolabeling in SNB motoneurons. The retrograde transport of BDNF is activity-dependent (Watson et al., 1999), and castration decreases activity in the SNB neuromuscular system (Fargo et al., 2003; Holmes and Sachs, 1992). Thus, this castration-induced decrease in activity could potentially result in lower rates of the retrograde transport of target-derived BDNF, which could account for the accumulation of BDNF in the muscle and a concomitant decrease in SNB motoneuron BDNF protein following castration. Alternatively, androgen-dependent alterations in BDNF levels in SNB motoneurons could reflect changes in local transcription, translation, or trafficking of BDNF within SNB motoneurons.

It is clear that androgens regulate BDNF and trkB expression in SNB motoneurons and this interaction has important implications for the maintenance of SNB motoneuron morphology. Castration decreases BDNF mRNA and protein in SNB motoneurons, and if BDNF signaling promotes the maintenance of SNB motoneuron morphology, these reductions could underlie the somal and dendritic regression seen after castration. Paradoxically, treating gonadally intact males with trkB IgG, a fusion protein that interrupts BDNF action, resulted in a hypertrophy of SNB dendritic arbors (Verhovshek and Sengelaub, 2010b; Fig.

2), suggesting that BDNF may be part of a signaling cascade exerting a tonic restraint on SNB motoneuron morphology. Interestingly, castration markedly elevated BDNF protein in the SNB target musculature and treatment with trkB IgG prevented the typical castration-induced dendritic atrophy; dendrite lengths in these animals were similar to those of gonadally intact males treated with trkB IgG. This was the first demonstration that the dendritic arbors of the highly androgen-sensitive SNB motoneurons could be maintained in the absence of androgens, and further suggested that the elevated BDNF levels in muscle were responsible for regressive changes in SNB morphology (Verhovshek and Sengelaub, 2010b). Together, these results suggest that BDNF exerts regulatory effects on SNB dendrites, because when the actions of BDNF are blocked, dendritic hypertrophy occurs, and castration-induced somal atrophy is prevented.

This conclusion is consistent with other results that demonstrate BDNF can drive regressive processes that affect neuronal morphology. For example, BDNF inhibits dendritic growth in developing layer VI cortical neurons (McAllister et al., 1997), decreases dendritic complexity in retinal ganglion cells (Lom and Cohen-Cory, 1999), and can decrease dendritic length in neurons of the nucleus of the solitary tract (Martin et al., 2012). Although it is not known if direct application or overexpression of BDNF in SNB motoneurons would result in decreased dendritic lengths, our study demonstrates that blockade of BDNF signaling results in dendritic hypertrophy in SNB motoneurons, suggesting that BDNF has a regulatory role in the maintenance of SNB morphology. Future studies should assess the effects of BDNF treatment on SNB morphology, and determine the site of action for BDNF regulation of SNB dendrogenesis.

Alternatively, trkB IgG may not cross the blood-brain barrier, and thus could be acting only peripherally to block BDNF signaling in the target muscles. SNB motoneurons could respond to the loss of peripheral BDNF with a compensatory increase in BDNF expression centrally to promote SNB dendrite growth. However, such an increase in BDNF expression in SNB motoneurons would have to be through an androgen-independent pathway, as dendritic hypertrophy after treatment with trkB IgG was seen in both gonadally intact and castrated males (and castration decreases BDNF protein and message in SNB motoneurons; Osborne et al., 2007; Ottem et al., 2007). Examination of BDNF protein and message expression in SNB motoneurons and their target musculature in trkB IgG-treated animals could address this critical outstanding question.

Although androgenic regulation of BDNF and trkB in SNB motoneurons and the morphological effects of BDNF blockade in adult rats have been well-documented, there is little research that has examined androgen-BDNF interactions on SNB motoneurons during development. Androgens play a critical role in the perinatal development of SNB motoneuron number: testosterone action at the SNB target musculature spares these motoneurons from normally-occurring cell death (Breedlove and Arnold, 1983; Freeman et al., 1996; Nordeen et al., 1985; Sengelaub and Arnold, 1986). Exogenous testosterone treatment in female rats prevents motoneuron cell death, resulting in a masculine SNB neuromuscular system (Breedlove and Arnold, 1983; Nordeen et al., 1985), presumably through androgen regulation of target-derived neurotrophic factors such as BDNF from the SNB target musculature. Indeed, blocking BDNF action at the SNB target musculature prevents the androgenic sparing of SNB motoneurons in newborn female rats (Xu et al., 2001). It is believed that androgens act at the target musculature to prevent SNB motoneuron death perinatally, possibly through an androgenic upregulation of BDNF in the target muscle that rescues motoneurons from cell death. The BDNF-producing capabilities of the developing SNB target muscles are not known, but because other skeletal muscles and corresponding motoneurons produce BDNF during development (Koliatsos et al., 1993; Griesbeck et al., 1995), it is possible that androgen-regulated, peripherally produced BDNF

is retrogradely transported to SNB motoneurons to promote survival during the perinatal period (Xu et al., 2001).

Target musculature

The SNB target musculature is critical for androgenic regulation of SNB morphology during development and in adulthood, and more recent work has explored BDNF and its androgen regulation in the SNB target muscles. BDNF mRNA and protein are present in skeletal muscle (Koliatsos et al., 1993; Kust et al., 2002; Mousavi et al., 2004) including the SNB target muscles (Verhovshek et al., 2010). BDNF levels in the SNB target musculature are regulated by androgens: in adult male rats, castration results in a significant increase in BDNF levels compared to gonadally intact animals, and treating castrates with testosterone reduces BDNF levels to those found in intact control males (Verhovshek et al., 2010; Fig. 3). Because both message and protein for BDNF are found in skeletal muscles, this suggests that the ability of androgens to regulate BDNF in skeletal muscle may reflect a change in the peripheral production of BDNF.

In addition to the androgenic regulation of BDNF levels in muscle, BDNF and androgens interact to influence gross muscle morphology as well. Blockade of BDNF signaling using trkB IgG resulted in hypertrophy of the SNB target musculature in gonadally intact male rats, and muscle weights were significantly greater for trkB IgG-treated animals compared to controls (Verhovshek and Sengelaub, 2010b). Similarly, trkB IgG treatment in castrated males attenuated the castration-induced decrease in SNB target muscle weight (Verhovshek and Sengelaub, 2010b). However, trkB IgG treatment did not maintain muscle weights at that of gonadally intact males, suggesting that although BDNF contributes to the regulation of SNB target muscle weight, other potentially androgen-sensitive factors are likely involved. These results represent the first demonstration that interfering with BDNF signaling can have trophic effects on skeletal muscle. Although the exact mechanism for BDNF's influence on skeletal muscle remains unclear, it has been suggested that BDNF plays an important role in the regulation of muscle homeostasis (Chevrel et al., 2006).

Somatic motor systems

Motoneurons

Androgenic regulation of BDNF and trkB in motoneurons is not restricted to androgen-sensitive neuromuscular systems. The non-dimorphic quadriceps motoneurons express BDNF and trkB, and the expression of this neurotrophic factor and its receptor are regulated by circulating hormones. In adult male rats, castration resulted in a significant decrease in both BDNF and trkB protein in quadriceps motoneurons (Osborne et al., 2007; Verhovshek et al., 2010). The castration-induced decrease in quadriceps motoneuron BDNF and trkB was restored to levels of those found in gonadally intact males by testosterone treatment (Osborne et al., 2007; Verhovshek et al., 2010).

Target musculature

In contrast to the SNB system, while BDNF levels in the quadriceps muscles are also regulated by androgens, castration reduces, rather than increases, BDNF protein in the muscle. BDNF protein levels are significantly lower in the quadriceps muscle of castrated adult male rats compared to gonadally intact animals, and treating castrates with testosterone restored BDNF levels to those of gonadally intact animals (Verhovshek et al., 2010; Fig. 3). In a potential naturally-occurring analog, BDNF mRNA in skeletal muscles is decreased in aged animals (Ming et al., 1999). Circulating androgen levels are also decreased in aged rats (Ghanadian et al., 1975; Kaler and Neaves, 1981), and thus, similar to castration effects in young adults, the decrease in BDNF mRNA in skeletal muscle could reflect age-related

decreased androgen. Alternatively, BDNF levels are activity-dependent, for example, increased after exercise (Gomez-Pinilla et al., 2001). Therefore, an age-related decrease in activity could be responsible for lower BDNF mRNA levels in the skeletal muscle of aged rats.

Summary of results

The pattern of androgenic regulation of trkB and BDNF in the highly androgen-sensitive motoneurons of the SNB is identical to that observed in the more typical somatic motoneurons innervating the quadriceps (Osborne et al., 2007; Verhovshek et al., 2010). In both systems, castration resulted in a decrease in trkB and BDNF protein levels in motoneurons, and testosterone treatment restored trkB and BDNF levels of those found in gonadally intact animals (Osborne et al., 2007; Verhovshek et al., 2010). In the SNB neuromuscular system, a site of action for androgenic regulation of BDNF in motoneurons is the target musculature (Verhovshek and Sengelaub, 2010a), but it has not been determined where androgens act to regulate BDNF expression in quadriceps motoneurons. Furthermore, the effect of interrupting BDNF signaling on quadriceps motoneurons should be assessed in order to compare the effects of BDNF blockade on motoneuron morphology across spinal motoneuron populations. Although these findings from quadriceps motoneurons suggest a general ability of androgens to regulate BDNF in spinal motoneurons, other somatic motor populations apparently do not display androgenic regulation of BDNF and trkB. In the retrodorsolateral nucleus (RDLN), a non-sexually dimorphic somatic motor population innervating foot muscles, castration had no effect on BDNF mRNA, protein, or trkB mRNA, and treating castrates with testosterone did not alter BDNF protein in proximal RDLN dendrites (Ottem et al., 2007). These contrasting results could simply reflect a difference between the RDLN and quadriceps motor populations. Alternatively, BDNF protein levels were measured 14 days post-castration in RDLN motoneurons compared to 28 days after castration in quadriceps motoneurons. Therefore it is possible that castration-induced changes in BDNF protein occur later in quadriceps compared to RDLN motoneurons. Alternatively, BDNF was measured in dendrites in the RDLN motoneurons, but in the somata of SNB motoneurons, and there could be differences in castration-induced changes in BDNF expression across cellular structures.

Androgens also regulate BDNF levels in target muscles in both the SNB and quadriceps neuromuscular systems. Interestingly, castration increased BDNF in the SNB target muscle, but decreased BDNF levels in the quadriceps, and in both systems, testosterone treatment in castrates restored BDNF levels to those of gonadally intact animals. Opposite effects of castration on BDNF concentrations in peripheral tissues have been previously observed. Castration decreased BDNF in the vas deferens but increased BDNF in the vesicular and prostate glands of rats (Mirabella et al., 2006). Together, these results demonstrate that within a neural system, androgen manipulation differentially affects BDNF levels in a structure-dependent fashion. Future studies should explore the role of steroid hormone-neurotrophic factor interactions in both sexually dimorphic as well as non-dimorphic populations of motoneurons.

The weights of the SNB and quadriceps motoneuron target muscles are also differentially sensitive to castration. In the SNB neuromuscular system, castration results in a decrease in target muscle weight, whereas quadriceps muscle weight remains unaffected after gonadectomy (Verhovshek et al., 2010). It is interesting to speculate that these changes could be potentially mediated by changes in BDNF levels in muscle. The increased levels of BDNF protein in the SNB target musculature after castration could underlie the decreases in muscle weight, and in fact trkB IgG treatment in castrates attenuates this decrease (Verhovshek and Sengelaub, 2010b). BDNF levels do not increase in the quadriceps muscle

after castration (in fact, they decrease; Verhovshek et al., 2010), potentially protecting the muscle from atrophy.

The differential androgen sensitivity of SNB target muscles and quadriceps muscles is likely mediated by differences in androgen receptor expression. Androgen receptors are present in substantially higher concentrations in the SNB target muscle compared to the quadriceps muscle (Dube et al., 1976). This difference in muscle androgen receptor density appears to confer androgen sensitivity on motoneuron morphology. Unlike SNB motoneurons, the morphology of quadriceps motoneurons is normally unaffected by androgen manipulation (Huguenard et al., 2011). As described above, BDNF and trkB protein in quadriceps motoneurons decrease after castration, and the lack of a concomitant decrease in dendritic length suggests that BDNF signaling does not promote dendritic growth in this system. However, androgen sensitivity can be induced in quadriceps motoneuron dendrites by increasing androgen receptor expression in the target musculature of transgenic rats (Huguenard et al., 2011). It would be interesting to examine if the pattern of changes in BDNF levels in response to androgen manipulations in the quadriceps system of androgen receptor overexpressing transgenic rats mimics that seen in the normally androgen-sensitive SNB neuromuscular system.

BDNF-androgen interactions in injury models

Androgens exhibit a wide array of neuroprotective and neurotherapeutic effects (Jones, 1993; Jones et al., 2001). For example, testosterone protects against cell death in cultured hippocampal neurons (Pike, 2001) and injury-induced dendritic atrophy in cortical pyramidal cells (Forgie and Kolb, 2003). In addition, neuroprotective and neurotherapeutic effects of androgens have also been demonstrated in motoneurons, including supporting cell survival, axonal regeneration, and dendritic maintenance (Fargo et al., 2009). The androgenic regulation of trophic factors has potentially important implications not only for adult maintenance of motoneuron morphology and function, but for neurotherapeutic or protective actions after motoneuron injury or disease (Fargo et al., 2009).

Axotomy

SNB motoneurons

BDNF-like immunoreactivity in SNB motoneurons is decreased dramatically after axotomy, suggesting that BDNF produced by the target muscles is retrogradely transported to SNB motoneurons (Yang and Arnold, 1998). Along with its androgen sensitivity, SNB motoneuron morphology is also influenced by BDNF. For example, BDNF regulates SNB soma size after axotomy: treatment with BDNF alone reversed the axotomy-induced SNB somal atrophy in castrated and gonadally intact animals (Yang and Arnold, 2000). Additionally, testosterone and BDNF interact to regulate several features of adult spinal motoneurons. For example, post-axotomy treatment with BDNF enhanced nuclear androgen receptor expression in SNB motoneurons, but only in the presence of testosterone (Yang and Arnold, 2000). In the absence of testosterone, BDNF treatment was ineffective, and androgen receptor immunoreactivity was comparable to the reduced levels found in castrated animals (Yang and Arnold, 2000). Similarly, following axotomy, combinatorial treatment with testosterone and BDNF maintained SNB motoneuron dendritic length in adult male rats; treatment with either testosterone or BDNF alone was ineffective (Yang et al., 2004; Fig. 4). The combinatorial treatment effects of testosterone and BDNF on androgen receptor expression and dendritic morphology in SNB motoneurons indicate that the neuromuscular periphery is a critical source of BDNF, as BDNF was applied directly to the cut axons (Yang and Arnold, 2000; Yang et al., 2004).

These findings demonstrate that BDNF and testosterone interact to provide trophic effects on SNB motoneuron morphology following axotomy (Yang et al., 2004). These results stand in strong contrast with the previously described inhibitory effects of BDNF on SNB dendritic morphology that occur in motoneurons with intact axons. This apparent conflict most likely reflects the major differences in the manipulations used in the two studies. For example, axotomy completely severs motoneuron contact with the periphery, whereas castration has no such effect. Indeed, motoneuron contact with the periphery is critical for androgen receptor and neurotrophin levels in SNB motoneurons: if cut axons are allowed to re-innervate their targets following axotomy, the axotomy-induced decrease in androgen receptor expression in SNB motoneurons is fully restored (Al-Shamma and Arnold, 1995). Additionally, other peripherally-derived signaling molecules may interact dynamically to regulate SNB dendritic morphology and by axotomizing SNB motoneurons, all of the potential effects of these various target-derived signals are disrupted. Indeed, axotomy alters the trophic factor responsitivity of motoneurons (Funakoshi et al., 1993; Koliatsos et al., 1994). On the other hand, blocking BDNF signaling with trkB IgG treatment may only have disrupted the BDNF component of this dynamic target-motoneuron interaction. Thus, it is likely that a variety of signaling pathways are differentially affected in these experimental manipulations, accounting for the different effects.

Peripheral nerve regeneration

Axons in the peripheral nervous system are known for their regenerative capacity, but recovery is protracted and often suboptimal (Fargo et al., 2009). A variety of factors have been identified that can be utilized to enhance nerve regeneration after nerve crush, including testosterone (Kujawa et al., 1989) and BDNF (Zhang et al., 2000). Relevant to this review, a few studies have examined the potential interaction of androgens and BDNF in peripheral nerve regeneration.

In *thy-1-YFP-H* mice, nerve grafts and exercise have been used as a treatment for peripheral nerve injury (Wood et al., 2011). After cutting a terminal branch of the sciatic nerve, male mice were subjected to treadmill training, and axon regeneration profiles were assessed at the end of the exercise treatment (Wood et al., 2011). Axon regeneration was enhanced by exercise, an effect likely mediated by an interaction of androgen and BDNF (Wood et al., 2011). Exercised mice had significantly greater levels of serum testosterone as well as increased BDNF and trkB mRNA in lumbar spinal cord compared to sedentary animals (Wood et al., 2011; Fig. 5). The effects of exercise on axon regeneration are dependent upon androgen receptor activation. For example, treatment with the androgen receptor blocker flutamide attenuated exercise effects on axon regeneration (Thompson et al., 2012). Similarly, exercise-induced enhancement of axon regeneration is also dependent on BDNF. Exercise had no effect on axon regeneration in BDNF-null mice (Wilhelm et al., 2012). These effects likely reflect an interaction of androgens and BDNF, as BDNF is increased immediately after peripheral nerve injury, and testosterone treatment further enhances and sustains BDNF mRNA levels, resulting in a long-term upregulation of BDNF in motoneurons (Sharma et al., 2010).

Mechanism of androgen regulation of BDNF

BDNF expression is regulated through a calcium-dependent signaling pathway, involving the phosphorylation of the cAMP response element (CRE) and its binding protein CREB (Shieh et al., 1998; Tao et al., 1998; Tao et al., 2002). Testosterone has been shown to activate both CRE and CREB (Aarnisalo et al., 1998; Auger et al., 2001) and thus, it is possible that the changes in BDNF immunolabeling in SNB motoneurons could involve an androgen-mediated regulation of the cAMP signaling pathway for BDNF. Alternatively,

many of the actions of testosterone occur through its conversion to dihydrotestosterone or estrogenic metabolites (Hutchinson, 1997), and thus it is possible that the effects of castration and/or testosterone replacement on BDNF immunolabeling could be either androgenic or estrogenic in nature. Estrogenic regulation of BDNF has been reported previously in several brain regions (Singh et al., 1995; Sohrabji et al., 1995; Gibbs, 1999; Jezierski and Sohrabji, 2001; Liu et al., 2001; Blurton-Jones et al., 2004) and although there is no evidence for estrogen accumulation in SNB motoneurons (Breedlove and Arnold, 1980), the SNB target musculature binds estrogens as well as androgens (Dube et al., 1976). Subsequent studies with estrogens or nonaromatizable androgens can address this question.

Implications of the androgen-BDNF interactions

This review highlights how androgens influence BDNF expression and motoneuron morphology by acting at the target musculature to regulate target-derived neurotrophic signals. Additionally, we provide evidence that BDNF's production, axonal transport, and androgenic regulation could be relevant for a variety of injury paradigms. Androgen-BDNF interactions may have relevance to therapeutic approaches in the treatment of neurodegenerative diseases or other human myopathies, as previous reports have suggested that abnormal expression of trophic factors and their receptors may play a role (Kust et al., 2002). It is important to note that in addition to its well-established trophic effects, BDNF can also have deleterious effects on neuron survival and morphology. For example, BDNF can render motoneurons vulnerable to excitotoxic insult *in vitro* (Fryer et al., 2000; Hu and Kalb, 2003; Mojsilovic-Petrovic et al., 2006) and has regressive effects on dendritic morphology *in vitro* (McAllister et al., 1997; Martin et al., 2012) and *in vivo* (Lom and Cohen-Cory, 1999).

In the case of neurodegenerative diseases, BDNF concentrations are elevated in human biceps muscles of patients with amyotrophic lateral sclerosis, a progressive motoneuron disease (Kust et al., 2002). Additionally, results of clinical tests using neurotrophic factors for the treatment of motoneuron disease are largely negative. Intrathecal treatment with recombinant methionyl human BDNF produced mild sensory symptoms, sleep disturbance, dry mouth, agitation and other behavioral effects requiring reductions in dosage (Ochs et al., 2000). Similarly, a Phase III multicenter clinical trial of ALS patients failed to show a benefit of BDNF treatment (The BDNF Study Group, 1999). Our recent evidence that blockade of excess BDNF production may be neuroprotective (Verhovshek and Sengelaub, 2010b) may be relevant for treating these types of diseases. Conversely, BDNF levels increase after testosterone treatment in multiple sclerosis patients, but this marked increase in BDNF occurs only in patients who have achieved full recovery, suggesting that heightened BDNF production is associated with positive outcomes for patients with certain diseases (Gold et al., 2008). Overall, it is possible that therapeutic strategies involving the regulation of trophic factors, rather than their simple administration, may be useful in treating degenerative neuromuscular diseases in which abnormal expression of trophic factors is a suspected cause.

Summary

This review has discussed studies demonstrating androgenic influences on BDNF signaling in neuromuscular systems. Further, we review evidence that suggests a mechanism for the trophic effects of androgens on spinal motoneurons via androgenic suppression of BDNF in the SNB target musculature. It is important to note that central upregulation of BDNF and trkB does not always indicate trophic effects on motoneuron morphology, and androgen-nregulated changes in BDNF protein and mRNA expression in neuromuscular systems should be directly evaluated. Also, we have compared androgen-BDNF interactions

following peripheral nerve injury in rodents. Results indicate that BDNF and androgens promote regeneration following axonal injury, potentially by an androgen-enhanced upregulation of BDNF in spinal motoneurons. Taken together, these findings provide further insight into the maintenance of neuromuscular systems in adulthood and have implications for the neurotherapeutic/neuroprotective roles of androgens and trophic factors in the treatment of motoneuron disease and recovery from injury.

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- We review androgen-BDNF interactions in the spinal cord.
- We describe these interactions following androgen manipulation and injury.
- Androgens interact with BDNF to regulate motoneuron death in development.
- In adulthood, androgens regulate BDNF and trkB expression in spinal motoneurons.
- In adulthood, androgens regulate BDNF levels in the target musculature.
- Androgen-BDNF interactions affect morphology, axon regrowth of injured motoneurons.

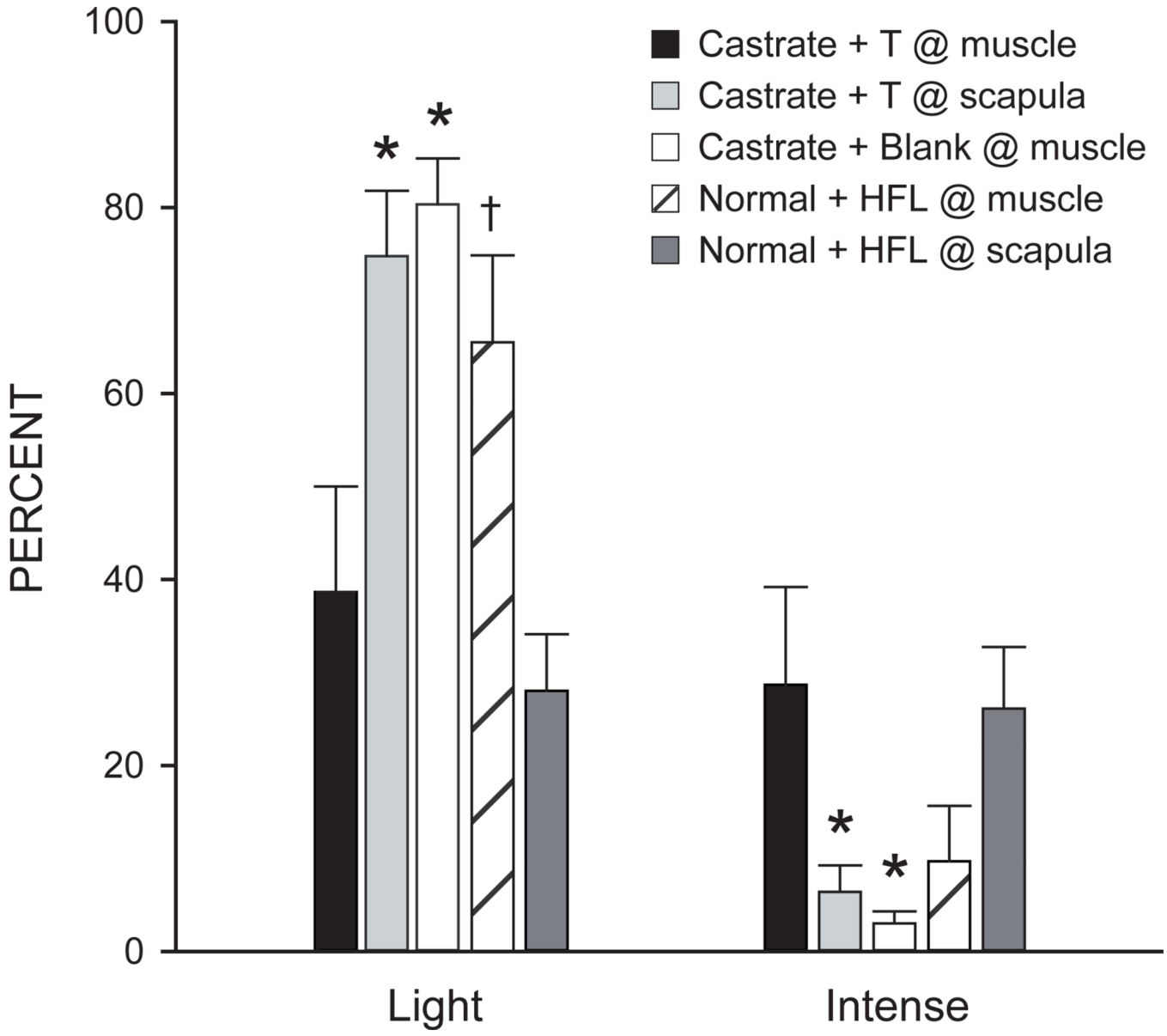

Figure 1.

Histogram of the number of lightly and intensely immunostained SNB motoneuron somata after immunolabeling for BDNF in castrated males with a testosterone (T) implant placed at the target muscle (black bars), castrated males with a T implant placed interscapularly (lightly shaded bars), castrated males with a blank implant placed at the target muscle (open bars), gonadally intact males with a hydroxyflutamide (HFL) implant placed interscapularly (hatched bars) and gonadally intact males with a hydroxyflutamide implant placed at the target muscle (darkly shaded bars). Bar heights represent means ± SEM. * Significantly different from castrated males with a T implant placed at the target muscle; † Significantly different from gonadally intact males with a hydroxyflutamide implant placed interscapularly. (Verhovshek et al., 2010a)

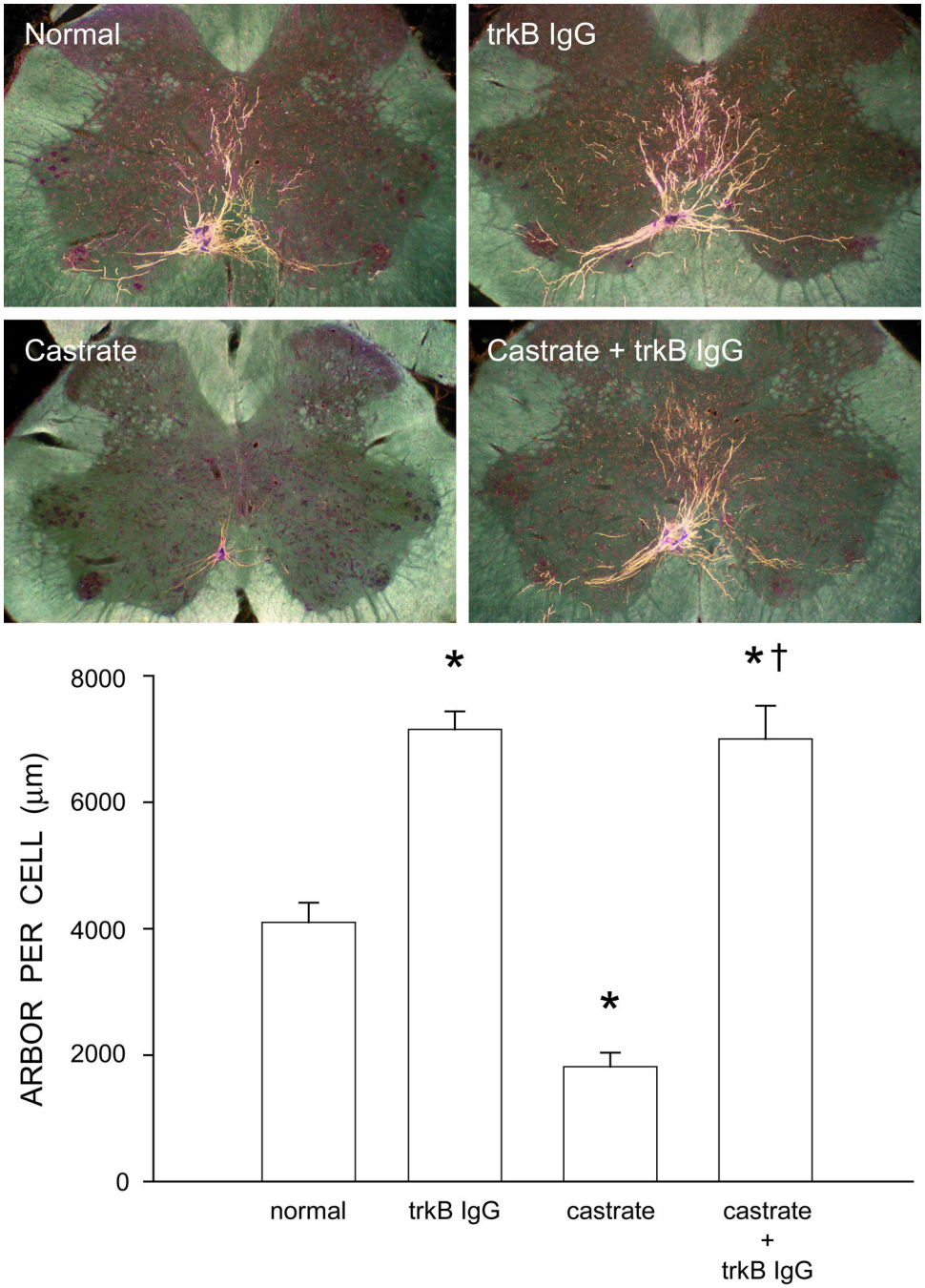

Figure 2.

(Top) Darkfield photomicrographs of transverse sections through the lumbar spinal cord of normal (top left), trkB IgG-treated (top right), castrated (lower left), and trkB IgG-treated castrated (lower right) males after unilateral BHRP injections into SNB target muscles. (Bottom) Dendritic length per labeled motoneuron in normal, trkB IgG-treated, castrated, and trkB IgGtreated castrated males. Bar heights represent means ± SEM. * Significantly different from normal males; † Significantly different from castrated males. (Adapted from Verhovshek and Sengelaub, 2010b)

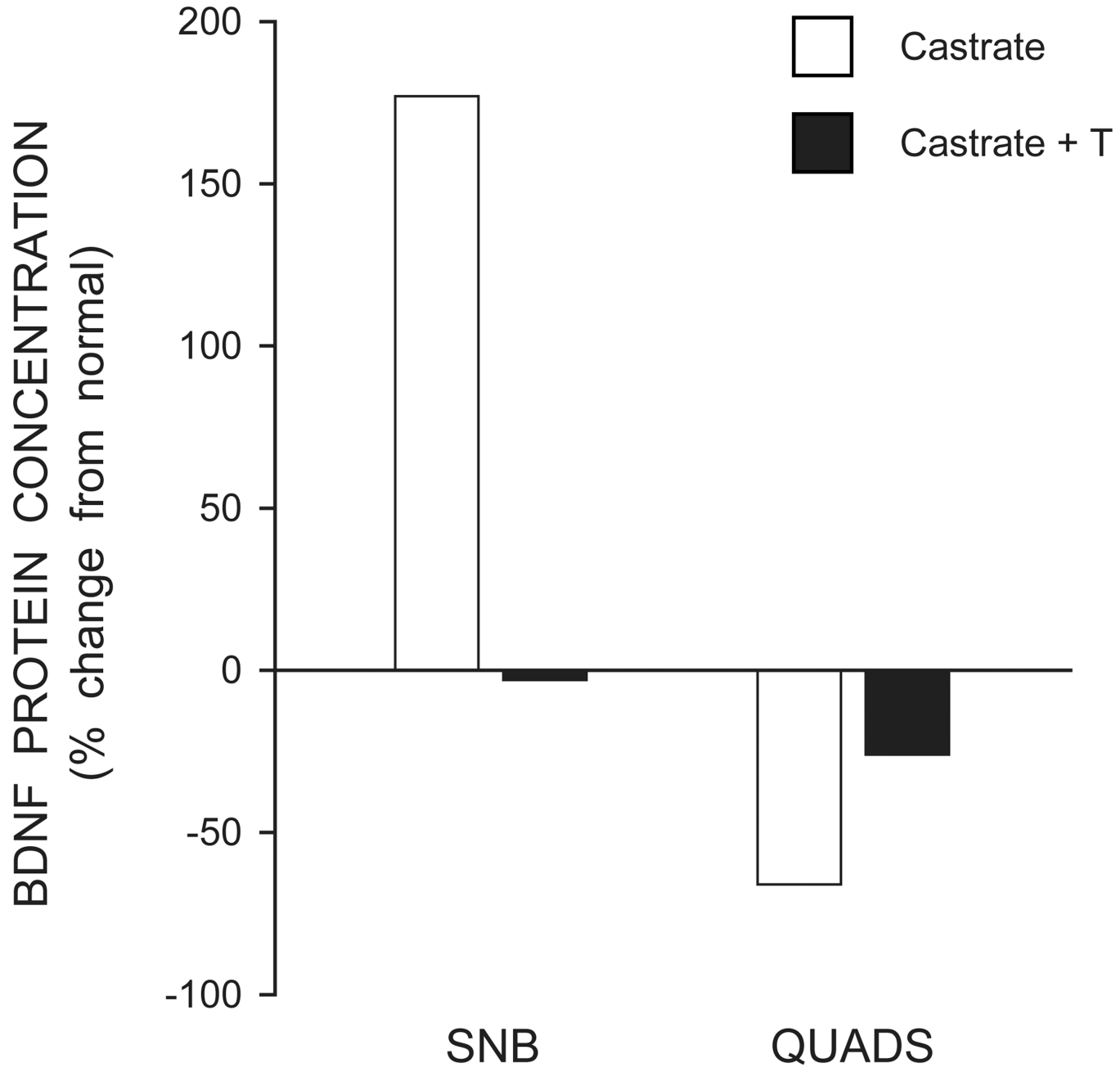

Figure 3.
BDNF protein concentrations in the target musculature of SNB (left) and quadriceps (QUADS, right) of castrated (open bars) and testosterone-treated (T) male rats (black bars). Concentrations are expressed as percent change from levels in gonadally intact males. (Adapted from Verhovshek et al., 2010)

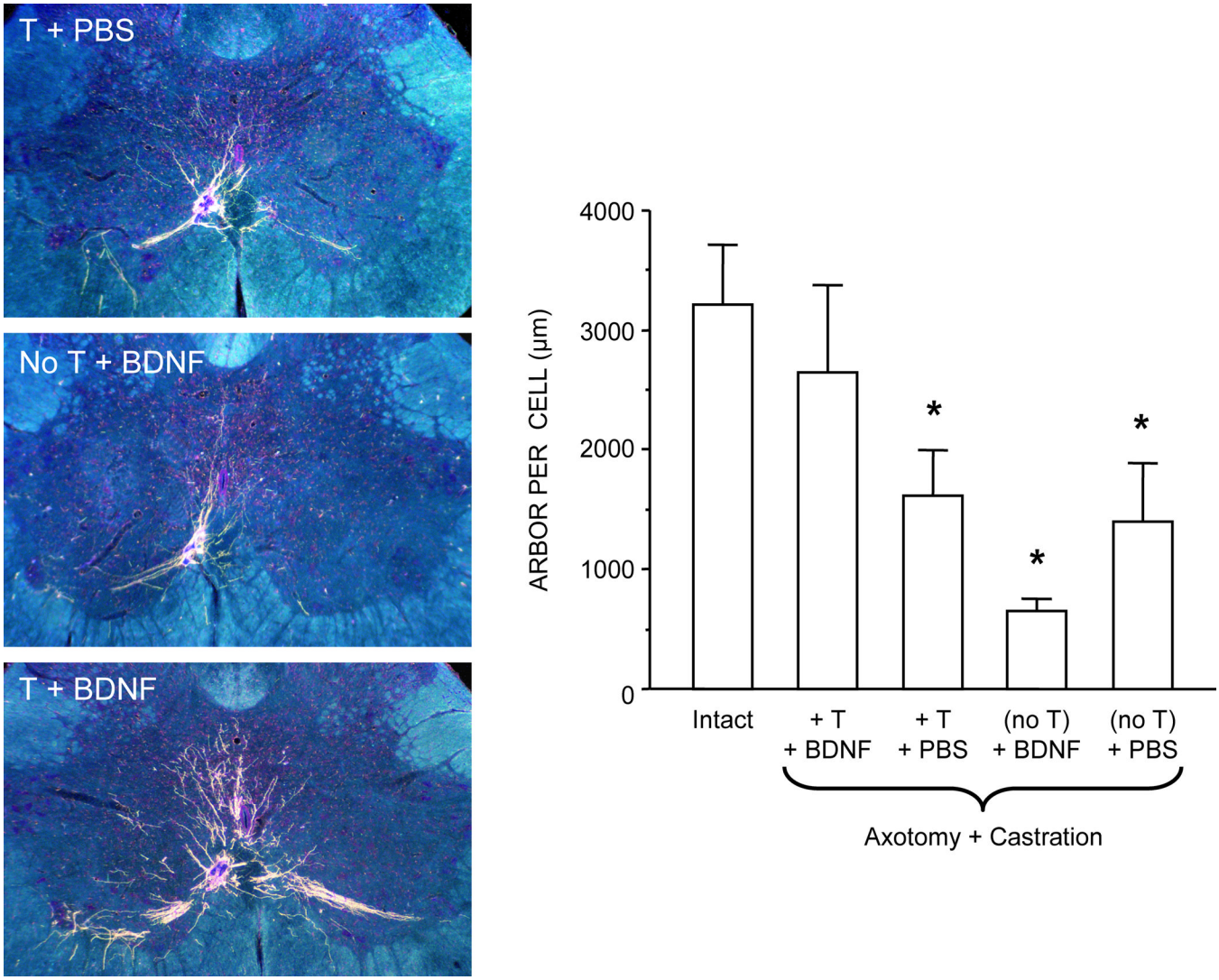

Figure 4.

(Left) Dark-field digital images of transverse sections through the lumbar spinal cord showing BHRP labeling of SNB dendrites in axotomized and castrated males treated with testosterone (T) and phosphate buffered saline (PBS) (top), BDNF alone (middle), or T and BDNF (bottom). (Right) Dendritic length per labeled motoneuron in intact males and axotomized and castrated males with T and BDNF (T+BDNF), T alone (T+PBS), BDNF alone (no T+BDNF), and PBS alone (no T+PBS) applied to the cut SNB axons. Bar heights represent means ± SEM. * Significantly different from intact males. (Adapted from Yang et al., 2004)

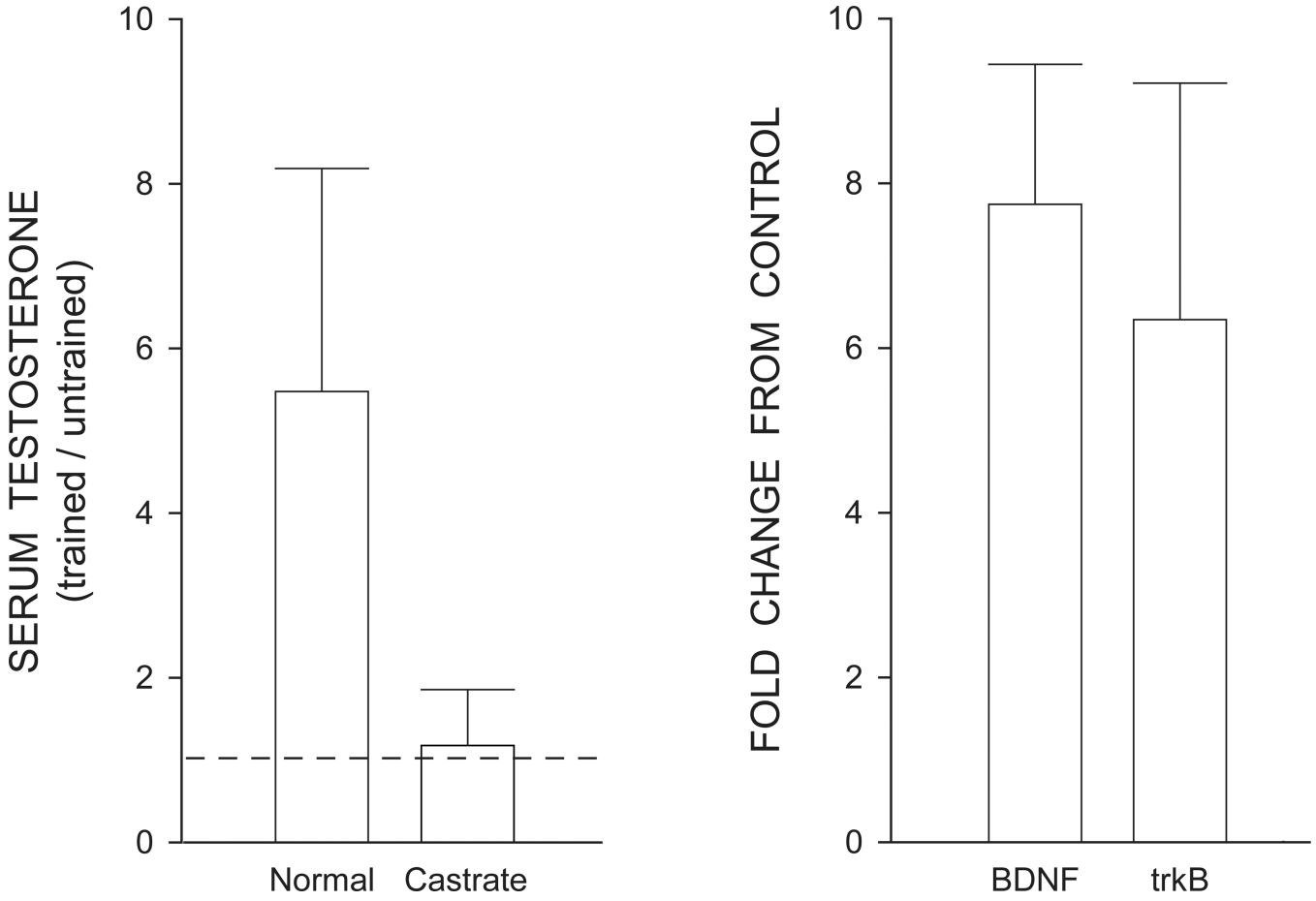

Figure 5.
(Left) Serum testosterone levels in treadmill trained normal and castrated male mice. Testosterone levels are expressed as the mean ratio of serum measurements in trained mice to untrained mice; dashed line indicates no change. (Right) BDNF and trkB expression in the lumbar spinal cords of gonadally intact treadmill trained male mice. Data are from real time PCR analysis expressed as mean fold change in BDNF mRNA relative to untrained mice. Bars in both histograms represent means ± 95% confidence intervals. (Adapted from Wood et al., 2012)