

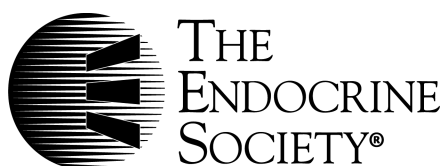
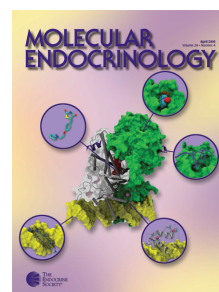
Endocrinology

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Brain-Derived Neurotrophic Factor and Androgen Interact in the Maintenance of Dendritic Morphology in a Sexually Dimorphic Rat Spinal Nucleus

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Testosterone regulates androgen receptor expression, soma size, and dendritic length in motoneurons of the spinal nucleus of the bulbocavernosus (SNB) in adult male rats. Brain-derived neurotrophic factor (BDNF) is also expressed in SNB motoneurons; BDNF maintains SNB soma size in castrates, and interacts with testosterone to regulate androgen receptor expression in SNB motoneurons. This study tested the hypotheses that BDNF promotes SNB dendritic lengths and that BDNF and testosterone interact to maintain dendritic morphology in SNB motoneurons. Adult male rats were castrated; and, 5 wk later, SNB motoneurons were axotomized bilaterally, and BDNF or PBS was applied via cups sutured to the cut axons. After axotomy plus BDNF or PBS application, castrates received implants of testosterone or blank capsules and were killed 24 d later. Additional males of similar age that received

sham castration, sham axotomy, and a blank implant served as sham controls. Two days before death, SNB motoneurons were retrogradely labeled with cholera toxin-horseradish peroxidase, and SNB dendritic morphology was reconstructed in three dimensions. Dendritic lengths in blank-implanted castrates treated with PBS were significantly shorter than those of sham controls; treatment with either testosterone or BDNF alone failed to support dendritic length or distribution. However, treatment with both testosterone and BDNF restored dendritic morphology to the level of sham controls. Our findings demonstrate that BDNF interacts with testosterone in the maintenance of SNB dendritic arbors and support the hypothesis that dendritic morphology is regulated by trophic substances that originate in the neuromuscular periphery. (*Endocrinology* 145: 161–168, 2004)

THE DENDRITIC ARBORS of spinal motoneurons are extensive, spanning several spinal segments and displaying elaborate local specializations and distributions. This elaborate arbor plays a critical role in motoneuron function, accommodating an estimated 20,000–50,000 synaptic inputs (1, 2). Differences in dendritic branching patterns, distribution, and overall shape determine important functional properties in motoneurons (3–7). For example, motoneurons innervating fast *vs.* slow fibers within the same muscle have different dendritic morphologies (4). Functionally, the morphology of spinal neurons correlates with their electrophysiological properties (8, 9). As a consequence, alterations in motoneuron dendritic morphology have a profound influence on motoneuron function, and understanding the mechanisms involved in the maintenance of motoneuron dendrites, or their restoration after injury, is of central importance.

Androgens regulate the motoneurons in a sexually dimorphic spinal nucleus of the bulbocavernosus (BC) (SNB) (10, 11). The medially located SNB contains approximately 200 and 60 motoneurons in the adult male and female rats, respectively (10, 11). In males, SNB motoneurons innervate the BC and levator ani (LA) muscles wrapping around the base

of the penis, and control penile reflexes important for copulatory behavior (10, 12). Androgens masculinize SNB motoneuron number and soma size (10, 11, 13). Androgens also regulate soma size (10, 11), the percentage of membrane contacted by glia (10), the number and size of synapses (10) and gap junction plaques (10), androgen receptor nuclear immunoreactivity (14–16), ciliary neurotrophic factor receptor α protein expression (17), calcitonin gene-related peptide (CGRP) mRNA and immunoreactivity (18–20), and mRNA expression of the cytoskeletal elements β -actin (21) and β -tubulin (22) in SNB motoneurons.

Dendritic morphology of SNB motoneurons is also regulated by androgens during development and in adulthood (10, 23). Dendritic development in the SNB is androgen-dependent. Dendrites typically grow profusely through the first 4 postnatal weeks, followed by retraction to adult lengths by 7 wk (23). In males castrated 7 d after birth, dendrites never grow beyond their precastration lengths, whereas dendritic lengths of castrates receiving testosterone replacement are equivalent to those of intact males by 4 wk of age (23). In adulthood, castration significantly decreases the dendritic lengths of SNB motoneurons in rats and mice, and testosterone replacement fully prevents or reverses this castration effect (10). Evidence further suggests that androgens can regulate SNB dendritic morphology by acting on the BC/LA muscles (24); in castrated males, SNB motoneurons projecting to testosterone-implanted BC/LA muscles have significantly longer SNB dendritic lengths than those projecting to muscles on the contralateral side implanted with hydroxyflutamide (an antiandrogen).

Brain-derived neurotrophic factor (BDNF) promotes den-

Abbreviations: BC, Bulbocavernosus; BDNF, brain-derived neurotrophic factor; BHRP, horseradish peroxidase conjugated to the cholera toxin B subunit; CGRP, calcitonin gene-related peptide; LA, levator ani; NMDA, N-methyl-D-aspartate; n.s., not significant; SNB, spinal nucleus of the bulbocavernosus.

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dratic branching in some types of neurons *in vivo* and *in vitro* (25–28) but has inhibitory effects on dendritic growth in others (29). Basal and apical dendrites of cortical pyramidal neurons grow substantially when exposed to exogenous BDNF *in vitro*, dramatically increasing dendritic length, branching, and the number of protospines (28). BDNF released from dendrites or cell bodies can increase dendritic growth of adjacent neurons *in vitro* (26). However, BDNF has also been shown to inhibit dendritic outgrowth; application of BDNF severely reduces retinal ganglion cell dendritic arbors, and antibodies to BDNF significantly increase dendritic lengths (29). Whether BDNF exerts a facilitative or an inhibitory effect on the dendritic morphology of SNB motoneurons still remains undetermined.

BDNF is expressed in SNB motoneurons and the BC/LA muscles and regulates the androgen receptor expression and soma size of SNB motoneurons. BDNF is present in SNB target musculature (BC/LA muscles) (30) and SNB motoneurons (31), and the BDNF-like immunoreactivity in SNB motoneurons is decreased dramatically after axotomy, suggesting that BDNF produced by the BC/LA muscles is retrogradely transported to SNB motoneurons (31). In addition to their sensitivity to androgens, SNB motoneurons are also affected by BDNF. Axotomy of adult SNB motoneurons causes a dramatic decline in the expression of androgen receptor nuclear immunoreactivity (16, 32–34). In the presence of testosterone, axotomy-induced loss of androgen receptor nuclear immunoreactivity in SNB motoneurons can be prevented or reversed by application of BDNF to cut SNB axons (16, 33, 34). Moreover, BDNF regulates SNB soma size; treatment with BDNF alone can reverse the axotomy- and castration-induced declines in SNB soma size (16).

Given that the morphology of dendrites in adult SNB motoneurons is androgen-dependent (10, 11) and that BDNF is expressed in SNB motoneurons (31) and affects dendritic outgrowth in several types of neurons, we hypothesized that BDNF promotes the dendritic arbors of SNB motoneurons and that BDNF and testosterone interact additively or synergistically to maintain SNB dendritic morphology. We tested these hypotheses by castrating male rats, applying BDNF or PBS to the cut SNB axons 5 wk later, implanting a sc testosterone or blank capsule immediately after BDNF or PBS application, and measuring the SNB dendritic arbors 24 d after BDNF or PBS treatment. Preliminary results of this study have been published in abstract form (35).

Materials and Methods

Animals

Young adult male rats (Sprague Dawley, Harlan Laboratories, Indianapolis, IN), 65–80 d old at the beginning of the study, were castrated and maintained on a 12-h light, 12-h dark cycle, with food and water freely available. Five weeks later, SNB motoneurons were axotomized bilaterally. Animals were anesthetized with sodium pentobarbital (55 mg/kg body weight, ip) and supplemented with Metofane (Pitman-Moore, Inc., Mundelein, IL). The SNB axons were exposed and cut on both sides at the location where they pass the bulbourethral gland and enter the BC/LA muscles. Immediately after axotomy, small SILASTIC (Dow Corning Corp., Midland, MI) brand cups (85–100 μ l in capacity) were sutured to the cut ends of the SNB axons. SILASTIC brand cups were made from medical grade SILASTIC, and consisted of a hollow cylinder (approximately 5 mm in diameter and 8 mm long) with an opening at one end and containing pieces of gelfoam. Each cup also

contained either 75 μ l recombinant human BDNF (generously provided by Regeneron Pharmaceuticals Inc., Tarrytown, NY; 5.8 mg/ml) or an equal volume of PBS (0.1 M, pH 7.4) delivered by a Hamilton syringe. This dose of BDNF has been found previously to produce maximal effects on SNB androgen receptor expression (34) and soma size (16). Immediately after axotomy and BDNF or PBS application, half of the animals received SILASTIC brand capsules (0.062 in. inside diameter $\times$ 0.125 in. outside diameter; 55 mm in length, Dow Corning Corp.) containing testosterone (4-Androsten-17 β -OL-ONE, Steraloids, Wilton, NH, filling 45 mm of the capsule length) made according to the procedures published by Smith *et al.* (1977) (36). The remaining rats received an empty SILASTIC brand capsule. To ensure sufficiency of BDNF until the end of the experiment, axotomized animals received a second identical injection of BDNF or PBS to the SILASTIC brand cup, 12 d after the first application (16). Additional males, which received sham castration, sham axotomy, a blank implant, and sham injections (the SNB axons were exposed but left untouched) at the corresponding times indicated above, served as sham controls. The resulting five treatment groups had four to six animals in each group. The research was conducted in our laboratories in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals. All surgeries were performed under sterile conditions.

Histochemistry

Horseshadish peroxidase conjugated to the cholera toxin B subunit (BHRP; List Biological, Campbell, CA) was used to retrogradely label SNB motoneurons. Previous studies have demonstrated that BHRP labeling permits sensitive detection and quantitative analysis of SNB somal and dendritic morphologies (23, 37). Ten days after the second application of BDNF or PBS, BHRP (1.0 μ l, 0.2%; List Biological) was injected unilaterally into the cut nerve and gelfoam stump inside the SILASTIC brand cup in each animal. In the control group, sham-operated males received unilateral injections of BHRP into both the BC and LA muscles (0.5 μ l each muscle) 57 d after sham castration.

Forty-eight hours after BHRP injection, a period that ensures optimal labeling of SNB motoneurons (23, 37), animals were overdosed with sodium pentobarbital (80 mg/kg body weight, ip) and perfused intracardially with saline followed by cold 1% paraformaldehyde/1.25% glutaraldehyde. Lumbar cords were removed, postfixed in the same solution for 5 h, and then transferred to sucrose phosphate buffer (10% wt/vol, pH 7.4) overnight for cryoprotection. Spinal cords were then embedded in gelatin and frozen-sectioned transversely at 40 μ m; all sections were collected into four alternate series. For visualization of BHRP, the tissue was immediately reacted using a modified tetramethyl benzidine protocol (38), mounted on gelatin-coated slides, and counterstained with thionin.

Dendritic length

Counts of labeled motoneurons in the SNB were made under bright-field illumination, where somata and nuclei could be visualized and cytoplasmic inclusion of BHRP reaction product could be confirmed. For each animal, dendritic lengths in a single representative set of alternate sections were then measured under dark-field illumination. Beginning with the first section where BHRP-labeled fibers were present, labeling through the entire rostrocaudal extent of the SNB dendritic field was assessed in every other section (320 μ m apart), in three dimensions, using a computer-based morphometry system (NeuroLucida, MicroBrightField, Inc., Colchester, VT; final magnification, $\times$ 250). Because the entire rostrocaudal range of the SNB dendritic field in each animal was sampled, this method allows for a complete assessment of SNB dendrites in both the transverse and horizontal planes. Average dendritic arbor per labeled motoneuron was estimated by summing the measured dendritic lengths of the series of sections, multiplying by 2 to correct for sampling, then dividing by the total number of labeled motoneurons in that series. This method does not attempt to assess the actual total dendritic length of labeled motoneurons (39), but has been shown to be a sensitive and reliable indicator of changes in dendritic morphology in normal development (23), response to hormonal manipulation (23, 37, 39, 40), and changes in dendritic interactions (41) or afferent input (42–44).

To assess potential redistributions of dendrites across treatment groups, for each animal, the composite dendritic arbor created in the

length analysis was divided using a set of axes radially oriented around the central canal. These axes divided the spinal cord into 12 bins of 30° each. The portion of each animal's dendritic arbor per labeled motoneuron contained within each location was then determined.

Dendritic extent

The rostrocaudal extent of the dendritic arbor was determined by recording the total rostrocaudal distance spanned by SNB dendrites, as well as the distances from where labeled fibers first appeared to the most rostral labeled motoneuron and from the most caudal labeled motoneuron to where the last fibers appeared for each animal. In the mediolateral plane, for each animal, the maximal radial extent of the dendritic arbor throughout the rostrocaudal extent of the SNB dendritic field was measured using the same radial axes and resultant 30° bins used for the dendritic distribution analysis. For each bin, the distance between the central canal and the most distal BHRP-filled process was measured in microns.

Statistics

Slides were coded, and all data were collected with no knowledge of treatment groups. Dendritic arbor per cell data (see Fig. 2) were analyzed by a one-way ANOVA followed by planned comparisons between each treatment and sham controls. Moreover, the main and interactive effects of BDNF and testosterone on dendritic length per cell (see Fig. 2) were examined by a two-way factorial ANOVA with hormone and trophic substance as factors followed by planned comparisons. For analyses of arbor distribution (see Fig. 3) and radial extent (see Fig. 4), comparisons among different treatments were performed by using a two-way repeated measures (group $\times$ location, with location as the repeated factor) followed by planned comparisons between each treatment and sham controls. An α -level of 0.05 was used for all statistical analyses.

Results

Injection of BHRP into either the axotomized nerve and gelfoam stump inside the SILASTIC brand cups or directly into the BC/LA muscles successfully labeled ipsilateral SNB motoneurons of all animals (35.81 ± 4.18 per animal; mean $\pm$ SEM) in a manner consistent with previous studies (23, 37, 45–47). SNB motoneurons displayed their characteristic multipolar morphologies, with dendritic arbors projecting ventrolaterally, dorsomedially, and across the midline into the area of the contralateral SNB (Fig. 1).

Dendritic length

The length of SNB dendrites per labeled motoneuron differed across groups [$F(4,20) = 3.78$, $P < 0.05$; Fig. 2]. SNB dendritic lengths in sham control males were typical of those of normal untreated males and did not differ from those of testosterone-implanted castrates who had BDNF applied to the cut nerves [$F(1,20) = 0.70$, not significant (n.s.)]. However, compared with sham control males, dendritic lengths were significantly reduced in blank-implanted castrates with either PBS or BDNF applied to their cut nerves, and testosterone-implanted castrates with PBS application [$F(1,20) > 4.36$, $P < 0.05$]. A two-way ANOVA (hormone treatment by trophic factor treatment, excluding sham control males) was also performed to assess potential independent effects of testosterone or BDNF treatment. No main effects of either hormone or BDNF treatment on SNB dendritic lengths were observed [$F(1,15) < 4.26$, n.s.]. However, testosterone treatment in animals who had BDNF applied to the cut nerves resulted in significantly longer SNB dendritic lengths than those of blank-implanted castrates [$F(1,15) = 6.82$, $P < 0.05$]. In con-

trast, testosterone treatment had no effect on dendritic lengths in castrates with PBS applied to the cut nerves [$F(1,15) = 0.12$, n.s.]. Similarly, BDNF application had no effect on dendritic lengths in blank-implanted castrates [$F(1,15) = 0.74$, n.s.].

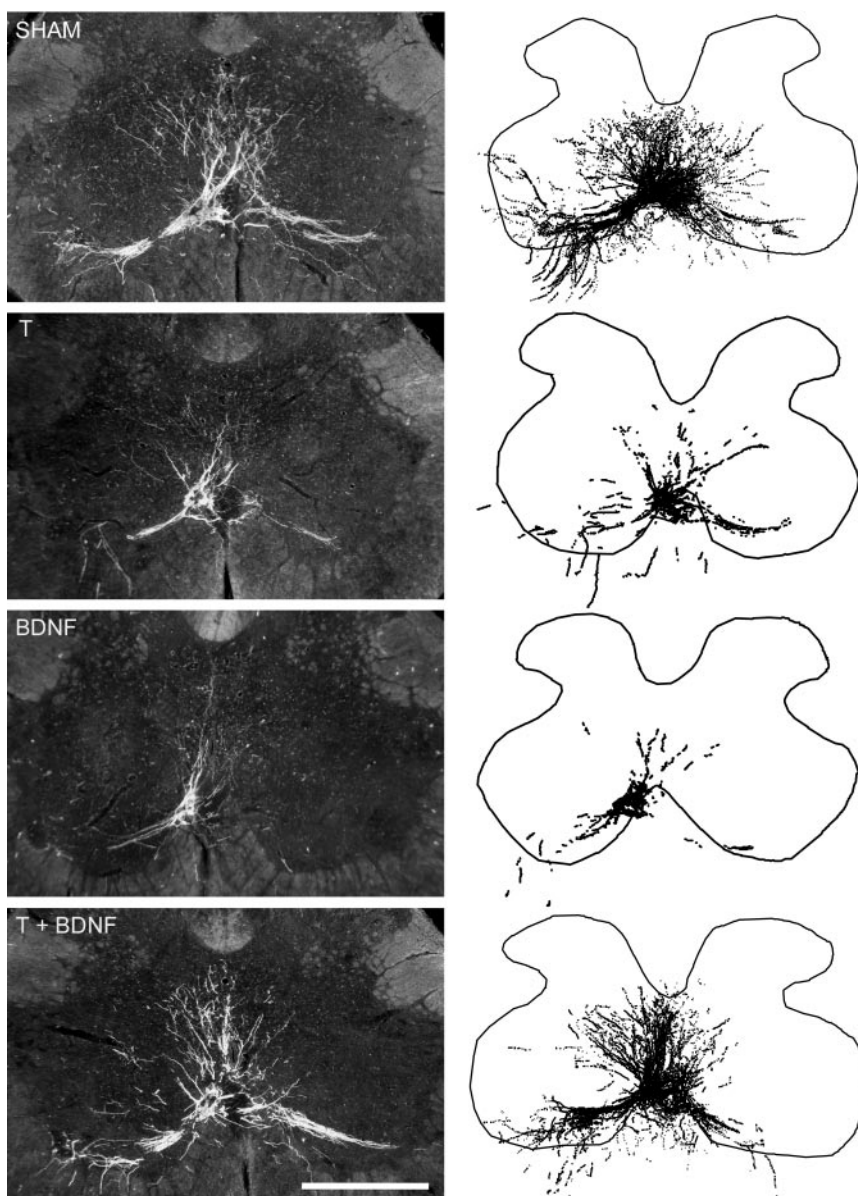
Dendritic distribution

As previously noted (41), the SNB dendritic arbor of normal males is radially organized but not uniformly distributed, with over 50% of the arbor concentrated ventrolaterally between 180° and 300° (Fig. 3). The distribution of SNB dendrites showed the typical effects of location [repeated measures $F(11,220) = 99.83$, $P < 0.0001$], as well as a main effect of group [repeated measures $F(4,220) = 3.95$, $P < 0.05$]. Compared with sham control males, treatment with both testosterone and BDNF supported the distribution of SNB dendritic arbor after axotomy [location by group interaction $F(11,110) = 1.92$, n.s.]. Although the nonuniform distribution of dendrites was retained after castration and axotomy, compared with sham control males, the amount of dendritic arbor in all locations was reduced in blank-implanted castrates with either PBS [ranging from 38% to 69% per bin; $F(1,99) = 8.55$, $P < 0.05$] or BDNF [60–93% per bin; $F(1,88) = 18.28$, $P < 0.01$] applied to their cut nerves, and testosterone-implanted castrates with PBS application [35–72% per bin, $F(1,88) = 5.56$, $P < 0.05$]. The distributions of dendritic arbor were not different among these three groups [location by group interaction, $F(22,110) = 1.44$, n.s.], indicating that treatment with either testosterone or BDNF was ineffective in reversing dendritic reductions at any location in the arbor.

Dendritic extent

The total distance spanned by SNB dendrites throughout the rostrocaudal axis did not differ across treatment groups [$F(4,20) = 2.20$, n.s.]. To rule out potential differences in the distribution of labeled motoneurons that could obscure group differences in the rostrocaudal extent of SNB dendrites, we also assessed the distances from where labeled fibers first appeared to the most rostral labeled motoneuron, and from the most caudal labeled motoneuron to where the last fibers appeared. As for total rostrocaudal extent, no local differences in dendritic extent at either the rostral or caudal limits of the arbor across groups were observed [$F(4,20) < 1.56$, n.s.]. In the mediolateral axis, a main effect of group was present in the overall radial extent of labeled dendrites [repeated measures $F(4,220) = 5.29$, $P < 0.001$; Fig. 4]. Planned comparisons revealed that radial extent did not differ in testosterone-implanted castrates who had BDNF applied to the cut nerves from that of sham control males [$F(1,110) = 1.19$, n.s.]. However, compared with sham control males, radial extent was reduced in blank-implanted castrates with either PBS [ranging from 8–54% per bin; $F(1,99) = 3.90$, $P < 0.05$] or BDNF [23–82% per bin; $F(1,88) = 34.74$, $P < 0.001$] applied to their cut nerves, and testosterone-implanted castrates with PBS application [8–40% per bin, $F(1,88) = 5.60$, $P < 0.05$]. The overall extent [$F(2,110) = 1.73$, n.s.] or radial pattern was not different among these three groups [location by group interaction, $F(22,110) = 0.94$, n.s.], indicating that treatment with either testosterone or BDNF alone was ineffective.

FIG. 1. *Left*, Dark-field photomicrographs of transverse sections through the lumbar spinal cord of a sham control male, an axotomized and testosterone (T)-implanted castrate who had PBS applied to the cut SNB axons, an axotomized blank-implanted castrate who had BDNF applied to the cut SNB axons, and an axotomized testosterone-implanted castrate who had BDNF applied to the cut SNB axons. *Scale bar*, 500 μ m. *Right*, Computer-generated composites of BHRP-labeled somata and processes drawn at 320- μ m intervals through the entire rostrocaudal extent of the SNB; these composites were selected because they are representative of their respective group average dendritic lengths.



fective in reversing reductions in dendritic extent at any location in the arbor.

Discussion

Castration dramatically decreases dendritic arbor, androgen receptor expression, and soma size of intact SNB motoneurons in adulthood, and testosterone replacement prevents or reverses these castration effects (10, 11, 14–16). In axotomized SNB motoneurons of castrates, testosterone markedly increases androgen receptor expression, and BDNF applied to the cut SNB axons further enhances this effect (16). A previous study, investigating the BDNF regulation of SNB soma size, found that castration and axotomy significantly reduces SNB soma size; application of BDNF to the cut SNB axons completely restores soma size to intact levels, either with or without testosterone replacement (16). In the present study, BDNF promoted dendritic growth of axo-

tomized SNB motoneurons in castrates, but this BDNF effect required the presence of testosterone to accomplish this task.

Comparability of BHRP labeling

Previous studies have demonstrated that neither axonal transport of BHRP (48) nor dendritic transport as demonstrated by the rostrocaudal or mediolateral extent of dendritic labeling (24, 39, 47) is affected by hormone levels. In the present experiment, the possibility that hormonal, surgical, or trophic factor manipulations could affect transport is an important consideration, because such artifacts could potentially result in an apparent shortening of dendritic length. Dendritic retraction after axotomy has been well documented using Golgi staining methodologies (see below) and thus was expected to occur in the present experiment; our data argue against additional apparent reductions in dendritic lengths or distributions because of hormone- or trophic

FIG. 2. Dendritic length per labeled SNB motoneuron in sham control males, axotomized and testosterone-implanted castrates who had BDNF (+T+BDNF) or PBS (+T+PBS) applied to the cut SNB axons, and axotomized and blank-implanted castrates who had BDNF (no T+BDNF) or PBS (no T+PBS) applied to the cut SNB axons. *Bar heights* represent means $\pm$ SEM for four to six animals per group. NS, $P > 0.05$; *, $P < 0.05$.

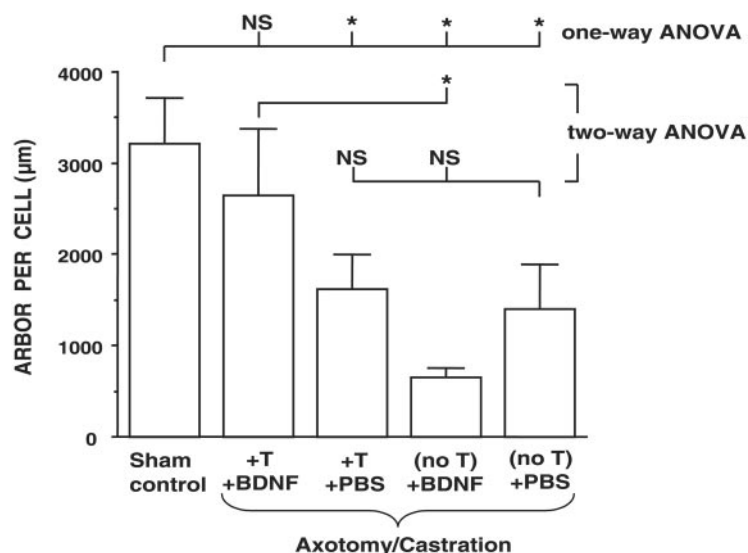
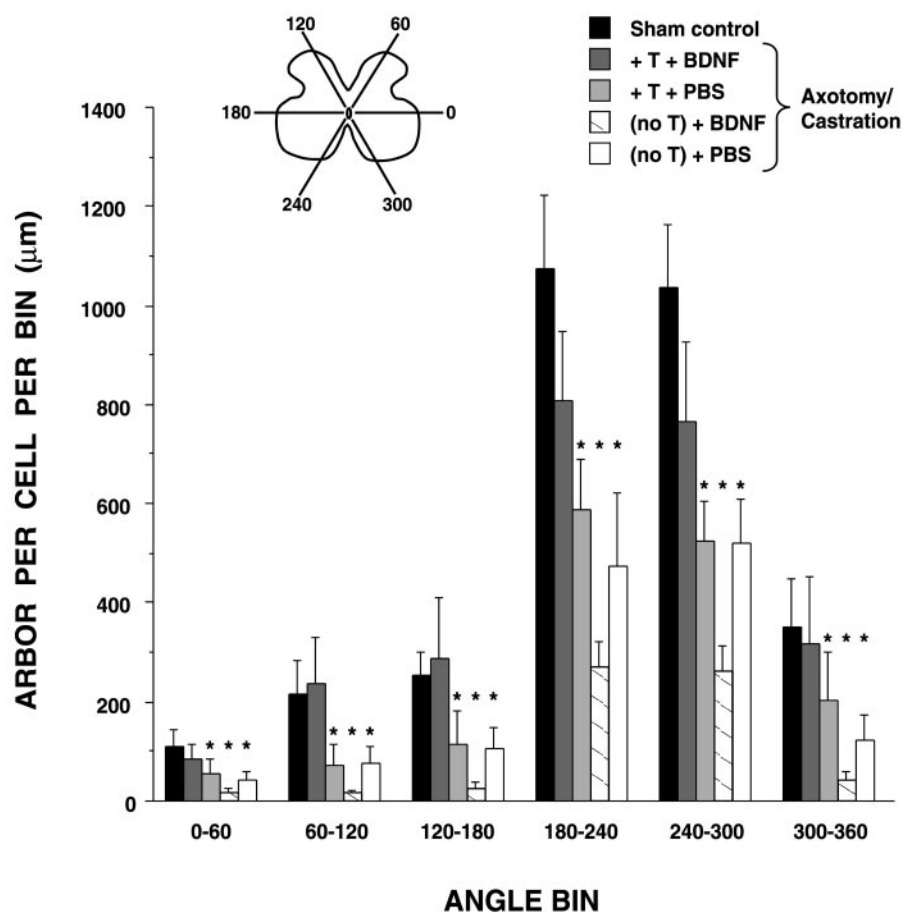


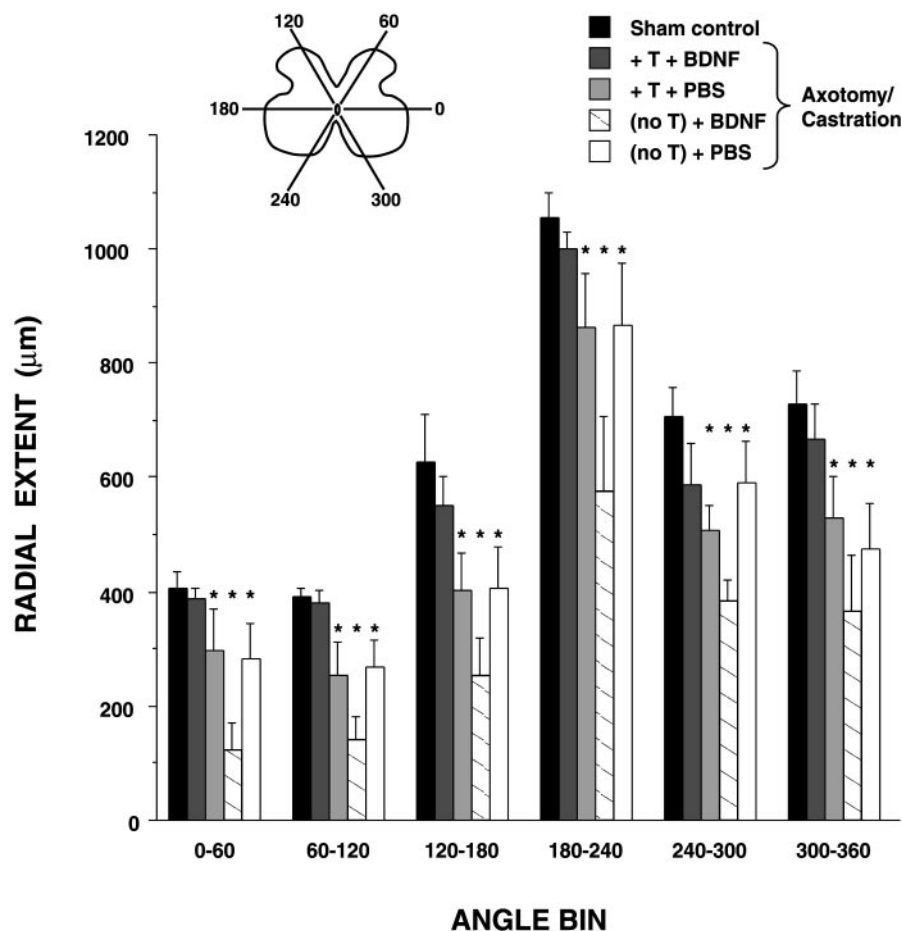
FIG. 3. Length per radial bin of SNB dendrites in sham control males (*filled bars*), axotomized and testosterone-implanted castrates who had BDNF (+T+BDNF; *heavily shaded bars*) or PBS (+T+PBS; *lightly shaded bars*) applied to the cut SNB axons, and axotomized and blank-implanted castrates who had BDNF (no T+BDNF; *hatched bars*) or PBS (no T+PBS; *open bars*) applied to the cut SNB axons; for graphical purposes, length per radial bin measures have been collapsed into six bins of 60° each. *Bar heights* represent means $\pm$ SEM for four to six animals per group; *, Significantly different from sham control males. *Inset*, Schematic drawing of spinal gray matter divided into radial sectors for measure of SNB dendritic distribution.



factor-induced transport artifacts. In the mediolateral plane, dendritic extent did not differ between testosterone- and blank-implanted castrates with PBS applied to their cut nerves, or between blank-implanted castrates treated with either BDNF or PBS, suggesting that differences in hormone or trophic factor levels had no effect on the ability of dendrites to transport BHRP. Furthermore, the rostrocaudal extent of dendritic labeling did not differ across groups, either

overall or at the rostral or caudal limits of the arbor, suggesting that if transport artifacts did occur, they would have to occur selectively in the transverse plane. Finally, the comparability of all measures between sham control males (in which SNB motoneurons were labeled after BHRP injection into the BC/LA muscles) and testosterone-implanted castrates treated with BDNF (in which SNB motoneurons were labeled after BHRP injection into the cut nerve and gel foam

FIG. 4. Radial extents of SNB dendrites in sham control males (filled bars), axotomized and testosterone-implanted castrates who had BDNF (+T+BDNF; heavily shaded bars) or PBS (+T+PBS; lightly shaded bars) applied to the cut SNB axons, and axotomized and blank-implanted castrates who had BDNF (no T+BDNF; hatched bars) or PBS (no T+PBS; open bars) applied to the cut SNB axons; for graphical purposes, dendritic extent measures have been collapsed into six bins of 60° each. Bar heights represent means $\pm$ SEM for four to six animals per group; *, Significantly different from sham control males. Inset, Schematic drawing of spinal gray matter divided into radial sectors for measure of SNB dendritic extent.



stump inside the SILASTIC brand cups) rules out transport artifacts attributable to axotomy alone. Thus, we believe the dendritic labeling across groups was comparable, allowing an accurate assessment of treatment effects on dendritic morphology after axotomy.

Dendritic response to axotomy

After axotomy, motoneurons show a range of responses, including structural, functional, and biochemical changes (*e.g.* Refs. 49–51). For example, axotomy of sciatic motoneurons by nerve crush causes dendritic retraction after 2 months (52). Axotomy also changes the electrophysiological properties of motoneuron dendrites, for example, giving rise to novel sodium-dependent partial spikes (53). Permanent axotomy of gastrocnemius motoneurons reduces dendritic diameter within 3 wk and dramatically decreases dendritic membrane area and volume within 12 wk (54). Actual disconnection of motoneurons from their target musculature is not required to induce dendritic retraction. For example, chemical blockade of functional contact between hypoglossal motoneurons and the tongue results in dendritic retraction (55). The dramatic regressions that occur in motoneuron dendritic arbors after axotomy can be reversed upon muscle reinnervation (52, 55, 56). This association between dendritic arbor size and muscle contact suggests that target musculature provides some sort of trophic support for motoneurons.

Neurotrophic effects on dendrites

In the SNB, very local effects seem to sculpt portions of developing dendritic arbors. For example, *N*-methyl-D-aspartate (NMDA) antagonism particularly alters the distribution of the dendritic arbor dorsolaterally (43), in the same areas where SNB premotor afferent interneurons have been identified (57). Similarly, the dendritic arbors of the two halves of the SNB overlap extensively, and experimentally induced reduction of this overlap early in development produces dramatic alterations in SNB dendritic morphology, especially in areas where dendrites from opposite sides of the nucleus would normally overlap (41). Furthermore, after spinal transection, the amount of SNB dendritic arbor located in the area where prominent projections from the lateral vestibular nucleus and gigantocellular reticular nucleus terminate (58, 59) is reduced by approximately 30% (42). In contrast to these very local effects, in the current experiment, the amount of SNB dendritic arbor was reduced in all locations throughout the arbor in blank-implanted castrates with either PBS or BDNF applied to their cut nerves, and testosterone-implanted castrates with PBS application. This uniform reduction suggests that a more general aspect of the regulation of dendritic morphology requires the interaction of testosterone and BDNF.

As stated previously, BDNF increases dendritic arborization in some types of neurons (25–28) but inhibits dendritic

growth in others (29). In axotomized SNB motoneurons of castrates, although we expected that BDNF treatment alone would exert a facilitative effect on dendritic arbors, we found that BDNF increased dendritic lengths only in the presence of testosterone.

The results from the current study indicated that the maintenance of SNB dendrites in adulthood was dependent on both androgen and trophic factors. As expected, SNB dendrites retracted substantially after castration and axotomy, reducing overall SNB dendritic lengths by over 50%; this decrease was distributed throughout the arbor. Furthermore, axotomy inhibited simple androgen effects in maintaining SNB dendrites after castration. Testosterone replacement, after castration, restores SNB dendritic length to normal adult levels (37); in the current study, dendritic retraction was not reversed in testosterone-implanted castrates with PBS applied to their cut nerves, suggesting that testosterone works with target-derived substance(s) to maintain dendritic morphology of SNB motoneurons. Both the BC/LA muscles and SNB motoneurons express BDNF protein, and axotomy dramatically decreases BDNF protein in SNB motoneurons (30, 31). In this study, we showed that application of BDNF to the cut SNB axons greatly increased the testosterone effect on SNB dendritic arbors. Together, these findings strongly suggest that target-derived BDNF is required for testosterone regulation of dendritic morphology of SNB motoneurons.

Rand and Breedlove (1995) (24) showed that testosterone can regulate SNB dendrites by acting at the target musculature. Local blockade of the androgen receptor, at the BC/LA muscles, with flutamide resulted in a 44% reduction in SNB dendritic length, suggesting that androgens regulate a neurotrophic signal from the muscle that is critical in the maintenance of dendritic organization. In the current study, treatment with either testosterone or BDNF alone failed to reverse axotomy- and castration-induced retractions of dendritic arbor. However, treatment with both testosterone and BDNF supported the dendritic morphology of axotomized SNB motoneurons in castrates. Because we applied BDNF peripherally to the cut nerves, our data support the hypothesis that SNB dendritic morphology is regulated by trophic substances from the neuromuscular periphery that are gated in their action by androgens.

Based on our current results and previous findings, we propose several possible mechanisms for the interaction of BDNF and testosterone in regulating SNB dendritic morphology. For example, it is possible that expression of BDNF by Schwann cells (60, 61) or the SNB target muscles (30) is sensitive to androgen. Thus, testosterone could act in the neuromuscular periphery to increase BDNF production, which in turn, enhances testosterone's effects either by increasing androgen receptor expression or by mechanisms independent of androgens or both.

Alternatively, the production of BDNF in the neuromuscular periphery may be independent of testosterone levels, but BDNF nonetheless could facilitate testosterone's effect on dendritic morphology of SNB motoneurons. Several lines of evidence indicate that neuronal activity increases BDNF production or transport. In cultures of rat hippocampal embryonic neurons, depolarization induced by high concentrations of potassium leads to a significant increase in BDNF mRNA

(62). Similarly, continuous KCl depolarization considerably increases BDNF release as detected by ELISA *in situ* techniques in primary cultures of rat nodose-petrosal ganglion cells (63). In cultures of cortical neurons, nuclear injection of cDNAs for green fluorescent protein (GFP)-tagged BDNF results in both anterograde and retrograde transport of BDNF (64). It is possible that SNB neuronal activity increases BDNF production in the BC/LA muscles and/or facilitates BDNF transport to SNB motoneurons. Consequently, the target-derived BDNF could work with testosterone to maintain the dendritic arbors of SNB motoneurons.

Another possible mechanism is that testosterone controls the expression of substances that promote the effect of BDNF on SNB dendritic arbors. Testosterone modulates several important biochemicals in the SNB, including ciliary neurotrophic factor receptor α (17), CGRP (18–20), β -tubulin (22), β -actin (21), and N-cadherin (65, 66), any of which could be involved in its interactive effects with BDNF. Moreover, application of trkB (the high-affinity receptor for BDNF) antagonist to the perineum blocked androgenic masculinization of SNB motoneuron number in newborn female pups treated with testosterone, suggesting that trkB mediates testosterone-increased survival of SNB motoneurons (67). Thus, it is quite likely that testosterone interacts with BDNF to maintain SNB dendritic morphology by regulating the expression of biochemicals mentioned above or increasing the trkB expression in the SNB motoneurons (68), which in turn, potentiates the response to BDNF transported from the neuromuscular periphery.

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