HHS Public Access

Author manuscript

Dev Neurobiol. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

Dev Neurobiol. 2017 June ; 77(6): 691–707. doi:10.1002/dneu.22445.

Neuroprotective effects of testosterone metabolites and dependency on receptor action on the morphology of somatic motoneurons following the death of neighboring motoneurons

Yi Cai, **Cory Chew**, **Fernando Muñoz**, and **Dale R. Sengelaub***

Program in Neuroscience and Department of Psychological and Brain Sciences Indiana University, Bloomington, Indiana 47405

Abstract

Partial depletion of spinal motoneuron populations induces dendritic atrophy in neighboring motoneurons, and treatment with testosterone is neuroprotective, attenuating induced dendritic atrophy. In this study we examined whether the protective effects of testosterone could be mediated via its androgenic or estrogenic metabolites. Furthermore, to assess whether these neuroprotective effects were mediated through steroid hormone receptors, we used receptor antagonists to attempt to prevent the neuroprotective effects of hormones after partial motoneuron depletion. Motoneurons innervating the vastus medialis muscles of adult male rats were selectively killed by intramuscular injection of cholera toxin-conjugated saporin. Simultaneously, some saporin-injected rats were treated with either dihydrotestosterone or estradiol, alone or in combination with their respective receptor antagonists, or left untreated. Four weeks later, motoneurons innervating the ipsilateral vastus lateralis muscle were labeled with cholera toxin-conjugated horseradish peroxidase, and dendritic arbors were reconstructed in three dimensions. Compared with intact normal animals, partial motoneuron depletion resulted in decreased dendritic length in remaining quadriceps motoneurons. Dendritic atrophy was attenuated with both dihydrotestosterone and estradiol treatment to a degree similar to that seen with testosterone, and attenuation of atrophy was prevented by receptor blockade. Together, these findings suggest that neuroprotective effects on motoneurons can be mediated by either androgenic or estrogenic hormones and require action via steroid hormone receptors, further supporting a role for hormones as neurotherapeutic agents in the injured nervous system.

Keywords

steroids; neuroprotection; morphology; dendrites

Neurodegenerative disease or injury often results in the loss of spinal motoneurons. For example, motor neuron diseases (e.g., amyotrophic lateral sclerosis; Cleveland and Rothstein, 2001) and spinal muscular atrophies (e.g., spinal and bulbar muscular atrophy; Kennedy et al., 1968) are characterized by progressive loss of motoneurons. Alternatively, damage to spinal nerves resulting in laceration and avulsion of spinal roots (e.g., cauda

*Correspondence to: Dr. D.R. Sengelaub, Department of Psychological and Brain Sciences, 1101 E. 10th Street, Indiana University, Bloomington, IN 47405. (812) 855-9149. FAX (812) 855-4691, sengelau@indiana.edu.

equina injury; Moschilla et al., 2001) can lead to the death of motoneurons and preganglionic autonomic neurons in the spinal cord, resulting in autonomic and motor dysfunction (Hoang et al., 2003).

Importantly, after such insults surviving motoneurons also show a variety of morphological and functional changes. For example, motoneurons undergo dendritic retraction and atrophy after injury (e.g., after spinal cord injury; Byers et al., 2012; Liu, 2014a,b). Similarly, after peripheral axotomy motoneurons show functional and biochemical changes (e.g., Titmus and Faber, 1990; Bisby and Tetzlaff, 1992) as well as dendritic atrophy (e.g., Sumner and Watson, 1971; O'Hanlon and Lowrie, 1995; Yang et al., 2004).

We have begun to examine the effects of motoneuron loss on the structure and function of surviving motoneurons using a rat model of motoneuron death. Our previous studies have demonstrated that surviving motoneurons respond to the loss of their neighbors with marked somal and dendritic atrophy (Fargo and Sengelaub, 2004a,b, 2007; Little et al., 2009). This secondary atrophy is responsible for at least some of the movement deficits that accompany degenerative movement disorders and spinal cord trauma, as it results in reduced excitability of the remaining motoneurons (Fargo et al., 2009b; Little et al., 2009). Given that we currently lack the technology to replace dead motoneurons, developing the ability to protect surviving motoneurons from secondary atrophy is an important goal.

Motoneurons display remarkable plasticity in adulthood and are capable of remodeling their cellular morphology; this propensity for change may allow for the development of treatment regimens in neural disease and injuries (Edgerton et al., 2004). Treatment with gonadal steroid hormones promotes a wide array of neuroprotective and neurotherapeutic effects (Jones et al., 2001; Fargo et al., 2009a; Foecking et al., 2015). For example, testosterone treatment accelerates both axon regeneration and functional recovery following axotomy of spinal or cranial motoneurons (Jones et al., 2001). After crush axotomy of hamster facial motoneurons, treatment with exogenous testosterone accelerates the rate of axon regeneration (Kujawa et al., 1991) as well as the recovery of motor function (Kujawa and Jones, 1990; Kujawa et al., 1989). Using our model of motoneuron loss, we have begun to examine the neuroprotective effects of gonadal steroids on the secondary atrophy induced in neighboring surviving motoneurons. Our previous experiments have demonstrated that testosterone is indeed neuroprotective following motoneuron loss. Treatment with exogenous testosterone attenuates the somal and dendritic atrophy, as well as the attenuated excitability, of motoneurons induced by motoneuron loss in both highly androgen-sensitive (Fargo and Sengelaub, 2004a,b, 2007; Fargo et al., 2009b) as well as more typical somatic (Little et al., 2009; Wilson et al., 2009) motoneuron populations.

The neuroprotective/neurotherapeutic effects of testosterone treatment may not necessarily be through the actions of testosterone per se, as testosterone is readily converted into its two primary metabolites, dihydrotestosterone via the enzyme 5α-reductase, or estradiol, by the enzyme aromatase. Both of these testosterone metabolites have been shown to have neuroprotective/neurotherapeutic effects (e.g., Garcia-Segura et al., 2000; Garcia-Segura et al., 2003; Huppenbauer et al., 2005; Barreto et al., 2007; Tanzer and Jones, 1997). However, in highly androgen-sensitive motoneurons, only treatment with androgenic hormones

protects remaining motoneurons from partial motoneuron depletion-induced dendritic atrophy, and estrogens are completely ineffective (Fargo and Sengelaub, 2007).

Gonadal steroids can exert their effects by acting as ligands for a subfamily of nuclear receptors that are generally thought of as transcription factors. Testosterone and dihydrotestosterone both activate androgen receptors, while estradiol activates estrogen receptors. In the presence of ligand, bound receptors translocate to the nucleus, interact with specific hormone response elements on genomic DNA, and increase or decrease transcription of steroid-regulated genes (Kumar and Thompson, 1999; Aranda and Pascual, 2001). Non-genomic actions of gonadal steroids have also been identified, and gonadal steroids and their cognate receptors may exert their effects through signaling pathways that do not directly or immediately involve transactivation of genomic DNA (Sen et al., 2011; Thomas, 2012; Frye, 2001; Lange et al., 2007).

The purpose of the present experiment was to determine the potential neuroprotective effects of the testosterone metabolites dihydrotestosterone and estradiol, as well as to assess their potential reliance on receptor-mediated mechanisms, in attenuating motoneuron atrophy after induced motoneuron depletion in the typical somatic motoneurons innervating the quadriceps muscle.

METHODS

In rats, the quadriceps muscle of the thigh comprises the vastus lateralis, the vastus medialis, the vastus intermedialis, and the rectus femoris (Hebel and Stromberg, 1986). These muscles are innervated by motoneurons located in column 3 of the lateral motor column in the L2 spinal segment (Nicolopoulos-Stournaras and Iles, 1983; Brushart and Seiler, 1987; Al-Majed et al., 2000) and project via the femoral nerve to the four muscles of the quadriceps. The distributions of the somata and dendritic arbors of the motoneurons innervating the individual muscles of the ipsilateral quadriceps overlap extensively in the lateral motor column (Nicolopoulos-Stournaras and Iles, 1983; Sengelaub et al., 2006), making it possible to partially and selectively deplete this motor population and study the effects of that depletion, and its potential hormone sensitivity, on the surviving motoneurons.

Animals

Adult male Sprague Dawley rats (approximately 100 days old; Harlan, Indianapolis, IN) were maintained on a 12:12 h light/dark cycle, with unlimited access to food and water. We used the toxin saporin, conjugated to the cholera toxin B subunit, to kill motoneurons selectively. Saporin is a ribosome-inactivating protein; it kills cells by irreversibly inactivating ribosomes and thereby halting protein synthesis (Stirpe et al., 1983, 1992). Cholera toxin-conjugated saporin is retrogradely transported from the site of injection, and kills motoneurons innervating the injected musculature within 3–6 days (Fargo and Sengelaub, 2004a).

Rats were anesthetized with isoflurane and motoneurons innervating the left vastus medialis muscle were selectively killed by intramuscular injection of cholera toxin-conjugated saporin (2 μl, 0.1%; Advanced Targeting Systems, Inc., San Diego, CA). Some of the rats

were not treated further (n = 6), whereas others were immediately treated with hormones with or without the appropriate receptor antagonist (see below).

To test if testosterone metabolites could be neuroprotective, some saporin-injected rats were treated with dihydrotestosterone (5α-androstan-17β-ol-3-one; Steraloids, Newport, RI; n = 7), administered via 30 mm long, subcutaneous, interscapular implants of Silastic tubing (Smith et al., 1977). Such implants produce plasma titers of dihydrotestosterone in the normal physiological range, and have previously been demonstrated to reduce motoneuron atrophy induced by the death of neighboring motoneurons in the highly steroid-sensitive spinal nucleus of the bulbocavernosus (SNB; Fargo and Sengelaub, 2007). Alternatively, some saporin-injected rats were treated with estradiol (1,3,5(10)-estratrien-3, 17β-diol; Steraloids; 10% in cholesterol; n = 5), administered via 10 mm long implants. Such implants generate serum levels of estradiol slightly higher than average peak estradiol levels in intact cycling females in the proestrous phase (Sfikakis et al., 1977; Overpeck et al., 1978), and completely prevent the effect of ovariectomy on uterine weight (Garrett and Wellman, 2009).

To test if steroid hormone receptor activation was required for neuroprotection, additional groups of hormone-treated saporin-injected animals were created. In one set, in addition to dihydrotestosterone implants, the nonsteroidal androgen receptor antagonist flutamide (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]-propanamide; Sigma-Aldrich, St. Louis, MO) was co-administered via 20 mm long implants (n = 5). Such implants are effective in blocking the intratesticular action of androgens (Porter et al., 2009). In another set of hormone-treated saporin-injected animals, in addition to estradiol implants, the nonsteroidal estrogen receptor antagonist tamoxifen ((*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethyl-ethanamine; Sigma-Aldrich) was co-administered via 45 mm long implants (n = 4). The dosage of tamoxifen was determined in pilot studies to be sufficient to reduce uterine weight in gonadally intact females to levels comparable to those of ovariectomized females. To control for potential effects of receptor antagonist treatment, two groups of normal animals were implanted only with flutamide (n = 3) or tamoxifen (n = 3) implants.

All implants were left in place until animals were euthanized. As in previous experiments (Fargo and Sengelaub, 2004a,b, 2007; Fargo et al., 2009b; Little et al., 2009; Wilson et al., 2009), animals in all saporin-injected groups were allowed to survive for 28 days following saporin injection, a length of time sufficient to observe saporin-induced effects on motoneuron morphology. Additional groups of age-matched, untreated animals served as normal controls (n = 5). Data collected previously from testosterone-treated saporin animals (n = 6; Little et al., 2009) were included for comparison (overall n = 43, 3–6 animals per group as detailed above).

Histological and histochemical processing

Four weeks after saporin injection, animals were reanesthetized, and the left vastus lateralis muscle (ipsilateral to the saporin-injected vastus medialis muscle in saporin animals) was exposed and injected with horseradish peroxidase (HRP) conjugated to the cholera toxin B subunit (BHRP; 2μL, 0.2%; List Biological Laboratories, Campbell CA). BHRP labeling permits population-level quantitative analysis of motoneuron somal and dendritic morphologies (Kurz et al., 1986; Goldstein et al., 1990). Forty-eight hours after BHRP

injection, a period that ensures optimal labeling of motoneurons (Kurz et al., 1986; Goldstein et al., 1990), animals were weighed, given an overdose of urethane (approximately 0.25 g/100 g body weight), and perfused intracardially with saline followed by cold fixative (1% paraformaldehyde/1.25% glutaraldehyde). To confirm the specificity of the saporin injections, the vastus medialis and the vastus lateralis muscles were removed bilaterally immediately after perfusion and weighed. The lumbar portion of the spinal cord of each animal was removed, postfixed in the same fixative for 5 hours, and then transferred to sucrose phosphate buffer (10% w/v, pH 7.4) overnight for cryoprotection. Spinal cords were then embedded in gelatin, frozen, and sectioned transversely at 40 μm; all sections were collected into four alternate series. One series was stained with thionin for use in cell counts. For visualization of BHRP, the three remaining series were immediately reacted using a modified tetramethyl benzidine protocol (Mesulam, 1982), mounted on gelatin-coated slides, and counterstained with thionin.

Motoneuron number and morphology

Motoneuron counts—Motoneurons innervating the quadriceps muscles do not form a discrete nucleus, but instead are contained within the large continuous populations of motoneurons located within the lateral motor column. Thus, to identify the appropriate area within the lateral motor column for motoneuron counts in the unreacted series, we used a method similar to that of Little et al. (2009).

Briefly, for each animal, the range of sections in which motoneurons labeled with BHRP after injection into the vastus lateralis muscle were present in the reacted series was identified. Motoneurons were then counted in the appropriate matching sections in the unreacted series. For each animal, estimates of the total number of motoneurons in the left and right lateral motor columns were obtained using the optical disector method as previously described (Little et al., 2009). Counts were made at ×937.5 under brightfield illumination. Quadriceps motoneurons are easily recognizable as large, darkly staining, multipolar cells.

A counting frame (110 × 80μm) was moved systematically throughout an area of each ventral horn (approximately 500 × 500μm, defined by the actual distribution of BHRP-labeled somata from all of the animals used in the study) in each section within the identified range. Only motoneurons in which there was a clear nucleus and nucleolus were counted, provided they did not contact the forbidden lines of the counting frame; motoneuron nucleoli were counted as they appeared while focusing through the z axis, and nucleoli in the first focal plane (i.e., “tops”) were excluded to avoid double counting. The length of the disector was approximately 16 μm, which was adequate for visualizing nucleoli in multiple focal planes. Motoneuron counts were derived from a mean of 17.1 sections spaced 160 μm apart and distributed uniformly through the entire rostrocaudal extent of the quadriceps motoneuron pool range. This sampling scheme produced an average estimated coefficient of error (CE) of 0.055. Cell counts for each animal were corrected for the proportion of sections sampled, and then expressed as a ratio (motoneuron number on the saporin-injected side relative to that on the untreated side) to quantify the magnitude of motoneuron depletion.

Using similar methods, the number of BHRP-filled motoneurons was assessed in all sections of the reacted series through the entire rostrocaudal extent of their distribution for all animals. Counts of labeled quadriceps motoneurons were made under brightfield illumination, where somata could be visualized and cytoplasmic inclusion of BHRP reaction product confirmed.

Soma size—The size of quadriceps motoneuron somata was assessed in at least one set of alternate sections (160 μm apart) by measuring the cross-sectional area of BHRP-filled motoneurons. Soma areas of an average of 20.3 motoneurons were measured for each animal using a video-based morphometry system (Stereo Investigator; MBF Bioscience, Williston, VT) at a final magnification of ×780. Soma areas within each animal were then averaged for statistical analysis.

Dendritic length—For each animal, dendritic lengths in a single representative set of alternate sections were measured under darkfield illumination. Beginning with the first section in which BHRP-labeled fibers were present, labeling through the entire rostrocaudal extent of the quadriceps motoneuron dendritic field was assessed; BHRP labeling spanned a rostrocaudal distance of an average of over 3200 μm. BHRP-labeled fibers were traced in every third section (480 μm apart) in three dimensions using a computer-based morphometry system (Neurolucida, MBF Bioscience) at a final magnification of ×250. Average dendritic length per labeled motoneuron was estimated by summing the measured dendritic lengths of the series of sections, multiplying by three 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 (Kurz et al., 1991), but it has been shown to be a sensitive and reliable indicator of changes in dendritic morphology in normal development (Goldstein et al., 1990, 1993; Goldstein and Sengelaub, 1993), in response to hormonal manipulation (Kurz et al., 1986, 1991; Forger and Breedlove, 1987; Goldstein et al., 1990; Goldstein and Sengelaub, 1993, 1994; Burke et al., 1997, 1999; Hebbeler et al., 2001, 2002; Hebbeler and Sengelaub, 2003), after changes in dendritic interactions (Goldstein et al., 1993) and afferent input (Kalb, 1994; Hebbeler et al., 2002; Hebbeler and Sengelaub, 2003), and after the death of neighboring motoneurons (Fargo and Sengelaub, 2004a,b; Little et al., 2009) or spinal cord injury (Byers et al., 2012).

Dendritic distribution—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 oriented radially around the center of the collective labeled somata. These axes divided the spinal cord into twelve bins of 30° each. The portion of each animal's dendritic arbor per labeled motoneuron contained within each location was then determined. This method provides a sensitive measure of dendritic redistribution in response to changes in dendritic interactions (Goldstein et al., 1993), afferent input (Hebbeler et al., 2002; Hebbeler and Sengelaub, 2003), or spinal cord injury (Byers et al., 2012).

Dendritic extent—The comparability of BHRP labeling across groups was assessed by quantifying both the rostrocaudal and the radial extent of quadriceps motoneuron dendritic arbors. The rostrocaudal extent of the dendritic arbor was determined by recording the

rostrocaudal distance spanned by labeled dendrites for each animal. The maximal radial extent of the arbor in the transverse plane was also measured for each animal, using the same radial axes and resultant 30° bins used for the dendritic distribution analysis: for each bin, the linear distance between the (should read:) center of the quadriceps motor pool and the most distal BHRP-filled process was measured. Radial dendritic extent is independent of overall dendritic length and reflects the maximal linear distance (in the transverse plane) of BHRP transport to the most distal dendritic processes.

All procedures were performed in accordance with Indiana University's Animal Care and Use Guidelines. All data were analyzed by *t*-test or analyses of variance (one way or repeated measures as appropriate), followed by *post hoc* analyses using Fisher's least significant difference (LSD). Digital light micrographs were obtained with an MDS 290 digital camera system (Eastman Kodak Company, Rochester, NY). Brightness and contrast of these images were adjusted in Adobe Photoshop (Adobe Systems, San Jose, CA).

RESULTS

Muscle weights

Differences in body weight were present across groups [$F(8,34) = 8.41$, $p < 0.001$], and thus raw muscle weights were corrected for body mass to assess potential effects of saporin and/or hormone treatment effects on muscle weight (Fig. 1). In normal animals, the corrected weights of the right (0.17 ± 0.01, mean ± SEM) and left (0.17 ± .01) vastus medialis muscles were similar [paired *t*-test, $t(4) = 2.14$, *ns*]. Although the weights of the uninjected (right) vastus medialis muscles were not affected [$F(8,34) = 1.81$, *ns*], unilateral injection of saporin into the left vastus medialis resulted in marked atrophy of the injected muscle across saporin groups [average 66.1% reduction in weight; $F(8,34) = 31.29$, $p < .0001$]. Compared to normal animals, saporin-injected animals had vastus medialis weights that were 74.1% lighter (LSD, $p < .0001$). Treatment with testosterone, estradiol, estradiol + tamoxifen, dihydrotestosterone, and dihydrotestosterone + flutamide did not prevent muscle weight loss across all saporin-treated groups (64.5% reduced; LSDs, ps < .0001 compared to normal animals) and did not differ from each other [$F(5,26) = 0.74$, *ns*]. Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on vastus medialis muscle weight (LSDs, *ns*).

Notably, the effect of saporin injection on quadriceps weight was specific to the injected muscle. In normal animals, the corrected weights of the right (0.40 ± .02) and left (0.41 ± .02) vastus lateralis muscles were similar [paired *t*-test, $t(4) = .43$, *ns*]. In the saporin groups, the weights of the vastus lateralis muscles on the untreated (right) side did not differ across groups [$F(8,34) = 1.07$, *ns*]. Most importantly, the weights of the vastus lateralis muscles adjacent to the saporin-injected vastus medialis muscles also did not differ across groups [$F(8,34) = 1.08$, *ns*]. Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on vastus lateralis muscle weight (LSDs, ns).

Motoneuron counts

In normal animals, the number of motoneurons within the identified quadriceps range did not differ between the left (251.2 ± 14.28) and right (237.6 ± 24.97) motor column [paired *t*-test, $t(4) = .63$, *ns*]. Motoneuron counts indicated that saporin was effective in inducing partial motoneuron depletion from the quadriceps motor pool (Fig. 2). Injection of saporin into the left vastus medialis muscle resulted in the death of ipsilateral quadriceps motoneurons, significantly reducing the number of motoneurons in the left motor column relative to that in the right [$F(8,34) = 4.57$, $p < .001$]. Unilateral injection of saporin into the left vastus medialis resulted in a 21.4% reduction in the relative number of motoneurons compared with that of normal animals (LSD, $p < .01$). Treatment with testosterone, estradiol, estradiol + tamoxifen, dihydrotestosterone or dihydrotestosterone + flutamide did not prevent this reduction (overall average of 25.3% reduced, LSDs, ps < .01 compared to normal animals); the reduced relative number of motoneurons within the quadriceps range did not differ across saporin groups [$F(5,26) = 1.43$, *ns*]. Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on motoneuron number (LSDs, ns).

Motoneuron morphometry

Injection of BHRP into the left vastus lateralis successfully labeled ipsilateral quadriceps motoneurons in all groups (Fig. 3). The dendritic arbor of quadriceps motoneurons was strictly unilateral, with extensive ramification along the ventrolateral margins of the gray matter and in the lateral funiculus, as well as throughout the ventral horn. An average of 34.60 (± 2.91) motoneurons per animal was labeled with BHRP, and this did not vary across groups [$F(8,34) = 1.79$, *ns*].

Soma size—Following saporin-induced motoneuron death, surviving neighboring quadriceps motoneurons underwent somal atrophy [$F(8,34) = 2.30$, $p <$. 05; Fig. 4]. Soma area in saporin-injected animals decreased by 15.7%; similar atrophies were present in the hormone-treated saporin groups (overall average reduction of 17.5%), and these did not differ from each other [$F(5,26) = 0.69$, *ns*]. However, only the reductions in soma sizes of testosterone- and estradiol + tamoxifen-treated saporin animals reached statistical significance (LSDs, ps < .05). Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on soma size (LSDs, ns).

Dendritic length—After saporin-induced motoneuron death, surviving neighboring quadriceps motoneurons underwent marked dendritic atrophy (Fig. 5). Dendritic length was decreased by 63.7% in saporin-injected animals compared to normal animals [LSD, $p <$. 0001; overall test for the effect of group on arbor per cell $F(8,34) = 11.90$, $p < .0001$]. However, whereas dendritic lengths in testosterone-, dihydrotestosterone-, and estradiol-treated saporin animals were also shorter than those of normal animals (LSDs, $p < .01$), hormone treatments attenuated induced dendritic atrophy, with dendritic lengths being reduced on average by only 39.66%. Compared with saporin animals given no supplemental hormones, testosterone-, dihydrotestosterone-, and estradiol-treated saporin animals had dendritic lengths that were 65.98% longer (LSDs, $p <$.04) and did not differ from each other (LSDs, ns). Treatment with receptor antagonists blocked the hormonal attenuation of

dendritic atrophy, with dendritic lengths being reduced by an average of 54.62% compared to normal animals (LSDs, $p < .001$); dendritic lengths in estradiol + tamoxifen- and dihydrotestosterone + flutamide-treated saporin animals did not differ from each other or those of saporin-only animals (LSDs, *ns*). Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on dendritic length (LSDs, ns).

Dendritic distribution—Dendritic length was non-uniform in radial bins, and a repeated-measures ANOVA revealed a significant effect of radial location [$F(11,374) = 25.27$, $p < 0.0001$; Fig. 6]. Consistent with the results in the arbor per cell analysis, there was also a significant effect of group [$F(8,374) = 11.99$, $p < .0001$]. Reductions in dendritic length occurred throughout the radial distribution, ranging from 38.2% (180° to 240°) to 78.5% (60° to 120°) in saporin-injected animals compared with normal animals [$F(1,99) = 26.19$, $p < .001$]. Treatment with testosterone, estradiol, and dihydrotestosterone in saporin animals attenuated these reductions, ranging from 26.0% (300° to 360°) to 74.2% (60° to 120°) compared with normal animals [$F(3,198) > 4.88$, $p < .05$]. Dendritic lengths per bin in hormone-treated saporin animals were longer than those of saporin-injected animals [$F(3,209) = 3.18$, $p < .05$] throughout most of the radial distribution, with increases ranging from 6.2% (120° to 180°) to 269.8% (0° to 60°). Although total dendritic lengths did not differ between hormone-treated saporin animals (see above), dendritic distributions in the transverse plane differed significantly [$F(22,154) = 3.26$, $p < .0001$]. Dendritic distributions were similar between testosterone- and estradiol-treated saporin animals [$F(11,99) = 1.54$, ns], but the distribution in dihydrotestosterone-treated saporin animals differed from both testosterone-treated [$F(11,110) = 3.09$, $p < .01$] and estradiol-treated [$F(11,99) = 5.50$, $p < .0001$] saporin animals, with shorter dendrites dorsomedially and longer dendrites ventrolaterally. Treatment with estradiol + tamoxifen and dihydrotestosterone + flutamide prevented the attenuation of saporin-induced dendritic atrophy seen with hormone treatment alone. Compared to normal animals, dendritic lengths in the hormone + receptor antagonist-treated saporin groups showed reductions ranging from 39.6% (300° to 360°) to 80.4% (120° to 180°) in arbor length post-saporin [$F(2,121) = 15.61$, $p < .001$]; dendritic lengths per bin did not differ from those of saporin-only treated animals [$F(2,132) = 0.99$, *ns*] or from each other [$F(1,77) = 0.40$, *ns*]. Treatment with receptor antagonists flutamide or tamoxifen in otherwise normal animals had no effect on dendritic distribution [$F(2,88) = 2.41$, *ns*].

Dendritic extent—In agreement with the nonuniform dendritic distribution of quadriceps motoneurons apparent in Fig. 3, radial dendritic extent differed across bins (Fig. 7), and a repeated-measures ANOVA revealed a significant effect of location [$F(11,374) = 28.43$, $p < .0001$]. However, radial dendritic extent did not differ across groups [$F(8,374) = 2.10$, *ns*]. Rostrocaudal dendritic extent also did not differ across groups [$F(8,34) = 1.69$, *ns*], spanning 3776.0 ± 546.3 μm in normal animals, 3760.0 ± 402.1 μm in saporin-injected animals, 3064.6 ± 219.7 μm in hormone-treated saporin animals, and 3066.7 ± 101.2 in otherwise normal animals treated with receptor antagonists.

DISCUSSION

Testosterone treatment protects both highly androgen-sensitive as well as typical somatic motoneurons from atrophy, and concomitant reductions in excitability, resulting from the death of neighboring motoneurons (Fargo and Sengelaub, 2004a,b, 2007; Fargo et al., 2009b; Little et al., 2009; Wilson et al., 2009). Testosterone can act directly as an androgen or be metabolized into other steroid hormones, both androgenic and estrogenic, but in highly androgen-sensitive motoneurons, only androgens are effective in protecting against dendritic atrophy following partial motoneuron loss (Fargo and Sengelaub, 2007). In contrast, in the present study, treatment with either androgens or estrogens was equally effective in attenuating dendritic atrophy after induced motoneuron depletion in the more typical somatic motoneurons that innervate the quadriceps motoneurons. Furthermore, both the androgenic and estrogenic effects on quadriceps motoneurons were prevented with receptor blockade, providing an important clue to the mechanism of neuroprotection.

Specificity and effectiveness of saporin injections

Muscle weights—Saporin injection into the vastus medialis significantly decreased muscle weight but had no effect on the adjacent, un-injected vastus lateralis muscle. These results are consistent with previous findings (Fargo and Sengelaub, 2004a,b; 2007; Fargo et al., 2009b; Little et al., 2009; Wilson et al., 2009) and indicate that saporin injections were effective at the targeted vastus medialis muscle and did not spread to the adjacent vastus lateralis musculature, an important consideration for interpreting the effects seen in surviving motoneurons.

Treatment with androgens or estrogens, or their respective receptor antagonists, had no effect on muscle weight. Hormonal manipulations did not prevent saporin-induced decreases in weight in the vastus medialis muscle; differences in the morphology of surviving motoneurons cannot thus be ascribed to differences resulting from the degree of peripheral damage. Similarly, hormonal manipulations had no effect on the weights of non-saporin-injected muscles; thus, the neuroprotective effects on the morphology of surviving motoneurons we observed are independent of muscle size alone. The absence of an effect on muscle weight with dihydrotestosterone treatment was expected and is consistent with our previous work in which we found that testosterone treatment had no effect on quadriceps muscle weight (Little et al., 2009; Wilson et al., 2009). Because the weight of the contralateral vastus lateralis muscles was not affected by androgen treatment, the lack of an androgen effect on the muscles adjacent to the saporin-injected muscles cannot be ascribed to an inhibition of an anabolic effect by saporin. Although androgens are known to have anabolic effects on general skeletal musculature, these effects are small in rats (Kochakian, 1975). The small anabolic effects, together with the modest physiological dosage of androgens used in the treatment groups, likely account for the lack of effects on quadriceps muscle weight we observed. The lack of effects in the estradiol manipulated groups was similarly expected, as estrogens do not affect skeletal muscle size (Fargo et al., 2003; Piccone et al., 2005).

Motoneuron Counts—Consistent with the results for muscle weights, motoneurons projecting to the vastus medialis were killed by intramuscular injection of saporin, reducing the number of motoneurons in the quadriceps pool by approximately 25% across the saporin-injected groups. Also consistent with the results for muscle weights, this induced death appears to have been specific to the motoneurons innervating the saporin-injected vastus medialis muscle, as there were no differences in the number of labeled motoneurons following injection of BHRP into the adjacent vastus lateralis. Blockade of androgen or estrogen receptors also had no effect on motoneuron number. Although steroid hormones have been shown to prevent injury-induced neuron death in other experimental models (Ahlbom et al., 2001; Hammond et al., 2001; Pike, 2001; Ramsden et al., 2003; Huppenbauer et al., 2005), they are not effective in preventing saporin-induced motoneuron death (Fargo and Sengelaub, 2004a,b; 2007; Little et al., 2009). In the present study, saporin was equally effective in killing motoneurons in all saporin-injected groups, regardless of hormone treatment. Thus, the beneficial effects of steroid treatment on the morphology of neighboring surviving motoneurons cannot be attributed to a hormone-mediated attenuation of the ability of saporin to kill motoneurons.

Atrophy in surviving motoneurons

After the selective death of motoneurons induced by saporin injection, the morphology of surviving neighboring motoneurons showed atrophy in both soma size and dendritic length, consistent with our previous studies in this motoneuron population (Little et al., 2009; Wilson et al., 2009) and others (Fargo and Sengelaub, 2004a, 2004b, 2007). Saporin-induced motoneuron death resulted in modest reductions in soma size but pronounced dendritic atrophy in surviving nearby quadriceps motoneurons. Why these atrophic changes in surviving motoneurons occur is unclear, but one potential mechanism could be through the loss of afferent fibers from the saporin-injected muscle. Just as for motoneurons, transport of saporin to dorsal root ganglion cells results in their death-we have found losses of over 78% of dorsal root ganglion cells innervating the vastus lateralis muscle following intramuscular injection of saporin (unpublished results, this laboratory). However, loss of this afferent projection would be specific to the medial aspect of the dorsal horn (the terminal field for these fibers, Molander and Grant, 1987), but the dendritic atrophy we observed was not restricted to any specific portion of the dendritic distribution, suggesting a general effect on the quadriceps motoneurons rather than a loss of a specific afferent population (Hebbeler and Sengelaub, 2003; Byers et al., 2012). Furthermore, the neuroprotective effects seen with hormone treatment cannot be attributed to a sparing of afferent fibers, as dorsal root ganglion cell death after intramuscular saporin injection does not differ between animals with or without testosterone treatment (unpublished results, this laboratory).

Alternatively, it is possible that a pronounced response of microglia to the partial depletion of motoneurons is responsible for atrophic changes in surviving motoneurons. We have recently shown that the total number of microglia, as well as the number of activated forms, in the area of the quadriceps pool is dramatically increased after saporin-induced motoneuron death (Kiley and Sengelaub, 2015). The atrophy observed in remaining motoneurons could be a result of this increased microglial activation in the injury site,

resulting in collateral damage through synaptic stripping and dendritic loss (Kettenmann et al., 2013).

Neuroprotection by androgens and estrogens

Soma size—Treatment with either androgens or estrogens, or their respective receptor antagonists, did not prevent reductions in quadriceps motoneuron soma size, consistent with the lack of effect of testosterone treatment previously reported (Little et al., 2009). The lack of effects of hormonal manipulations in quadriceps motoneurons is likely due to the lower hormone sensitivity in this neuromuscular system (Little et al., 2009). For example, castration with or without hormone replacement has no effect on soma size in quadriceps motoneurons (Osborne et al., 2007; Huguenard et al., 2011). In contrast, soma size in highly androgen-sensitive motoneurons responds dramatically to hormonal treatment; for example, castration results in dramatic reductions in soma size in the androgen-sensitive motoneurons of the SNB (up to 45%) which can be reversed with testosterone treatment (Kurz et al., 1986). Directly relevant to the current study, following partial motoneuron depletion of the highly androgen-sensitive SNB motoneurons, treatment with either testosterone or dihydrotestosterone attenuates induced somal atrophy in SNB motoneurons (Fargo and Sengelaub, 2004b, 2007). This effect is strictly androgenic, as treatment with estradiol is completely ineffective (Fargo and Sengelaub, 2007). These differences in hormonal response across motor populations likely reflect differences in their expression of androgen and estrogen receptors (see below).

Dendritic arbor—Consistent with the effect of testosterone treatment (Little et al., 2009; Wilson et al., 2009), treatment with androgens (either testosterone or dihydrotestosterone) attenuated induced atrophy of quadriceps motoneuron dendrites, and to comparable levels. Quadriceps motoneurons in testosterone- or dihydrotestosterone-treated saporin animals had dendritic lengths that were approximately 40% shorter than normal lengths (compared to over 60% in untreated saporin animals), and these were longer than those of untreated saporin-injected animals by approximately 65%. These results parallel those found in the highly androgen-sensitive SNB motoneurons, where treatment with either testosterone or dihydrotestosterone also attenuated induced dendritic atrophy in surviving neighboring motoneurons, albeit to a greater degree. In SNB motoneurons, treatment with testosterone or dihydrotestosterone attenuated induced dendritic atrophy, resulting in dendritic lengths that were less than 20% shorter than normal lengths, and these were longer than those of untreated saporin-injected animals by almost 140% (Fargo and Sengelaub, 2007).

Surprisingly, treatment with estradiol also attenuated induced atrophy in quadriceps motoneuron dendrites, and to a level similar to that achieved with androgen treatments. Quadriceps motoneurons in estradiol-treated saporin animals had dendritic lengths that were approximately 39% shorter than normal lengths, and these were longer than those of saporin-injected animals by approximately 67%. This effect of estradiol in quadriceps motoneurons is consistent with a large literature documenting the neuroprotective effects of estrogens (Henderson and Reynolds, 2002; Woolley and Cohen, 2002). For example, estradiol protects cortical and hippocampal neurons from cell death induced by ischemia (Hoffman et al., 2006), and promotes cell survival (Huppenbauer et al., 2005) and axonal

regeneration (Tanzer and Jones, 1997) in brain stem motoneurons following peripheral axotomy. However, the estrogenic effect we observed in quadriceps motoneurons stands in stark contrast to that found in the highly androgen-sensitive SNB motoneurons, where treatment with estradiol was completely ineffective in preventing dendritic atrophy after saporin-induced motoneuron death (Fargo and Sengelaub, 2007). As for soma size changes, these differences in hormonal response for dendritic lengths across motor populations likely reflect differences in their expression of androgen and estrogen receptors (see below). Furthermore, the success of treatments with dihydrotestosterone or estradiol suggest that the neuroprotective effects of testosterone we have observed previously in quadriceps motoneurons could in fact be mediated through its conversion to androgenic and estrogenic metabolites.

Although total dendritic lengths did not differ between hormone-treated saporin animals, hormone treatment differentially affected dendritic distributions in the transverse plane. Dendritic distributions were similar between testosterone- and estradiol-treated saporin animals, but the distribution in saporin animals treated with the nonaromatizable dihydrotestosterone were different, with shorter dendrites dorsomedially and longer dendrites ventrolaterally. This differential response suggests that the hormone metabolites act through different mechanisms/pathways to attenuate induced dendritic atrophy. For example, in dihydrotestosterone-treated saporin animals, the relative lack of dendritic protection dorsomedially (where muscle afferents terminate, Molander and Grant, 1987) suggests that estrogens might attenuate motoneuron dendritic atrophy via an interaction involving muscle afferents.

Previous studies have demonstrated that neither axonal transport of BHRP (Leslie et al., 1991) nor dendritic transport as demonstrated by the rostrocaudal or radial extent of dendritic labeling (Fargo and Sengelaub, 2004b; Goldstein and Sengelaub, 1994; Hebbeler et al., 2002; Kurz et al., 1991) are affected by hormone levels. Thus, in the present study, we believe that the differences we observed across treatment groups reflect dendritic atrophy in surviving motoneurons in saporin-injected animals, which is attenuated by treatment with hormones. The possibility that confounds arising from saporin injection could affect retrograde transport is also an important consideration, as such an artifact could potentially result in apparent alterations in dendritic morphology. However, no differences in either radial or rostrocaudal extents of quadriceps motoneuron dendrites in the saporin groups compared to normal values were observed. Therefore, we believe that the dendritic labeling across groups was comparable and that the shorter dendritic lengths we observed in saporin groups reflect dendritic atrophy.

The role of steroid receptors

Treatment of otherwise normal animals with androgen or estrogen receptor antagonists had no effect on quadriceps motoneuron morphology, suggesting that gonadal steroids are not involved in the normal maintenance of these features. However, receptor blockade in saporin-injected animals completely prevented the neuroprotective effects of androgen or estrogen treatment: quadriceps motoneurons in hormone + antagonist-treated saporin animals had dendritic lengths that were reduced to degrees comparable to those of untreated

saporin animals. This suggests that the protective effects of androgens and estrogens are mediated through the activation of their respective steroid receptors.

The differences in both the magnitude of the neuroprotective effects and the form (androgenic versus estrogenic) of gonadal hormone that produce them across motor populations potentially offer clues to the mechanisms involved. We have previously speculated that the difference in the magnitude of the protective effects of androgen treatment between quadriceps motoneurons and the highly androgen-sensitive SNB motoneurons lies in the different levels of expression of androgen receptors in these two systems (Little et al., 2009). All spinal motoneurons contain androgen receptors (Matsuura et al., 1993; Lumbroso et al., 1996), but SNB motoneurons abundantly express androgen receptors at levels far above typical somatic motoneurons (Breedlove and Arnold, 1980, 1983). Similarly, the principal SNB target muscles in males are enriched for androgen binding sites and androgen receptor protein compared to other striated muscles (Dubé et al., 1976; Tremblay et al., 1977; Monks et al., 2006). Thus, as differences in the density of androgen receptors are thought to underlie differences in androgen responsiveness across tissues (Monks et al., 2006), the lower density of androgen receptors in the quadriceps system might result in a smaller protective effect of testosterone.

The actions of testosterone and dihydrotestosterone are mediated by the same receptor (Chang et al., 1988a,b), and the presence of androgen receptors in both the motoneurons and the muscles they innervate suggests that these androgens could act at either site to mediate the neuroprotective effects we observed. However, this is not the case for estrogen receptors. Rat spinal motoneurons do not contain estrogen receptors (Breedlove and Arnold, 1980, 1983; Keeffer et al., 1973; Morrell et al., 1982; Simerly et al., 1990; Williams and Papka, 1996), but their target muscles can to varying degrees. For example, the target muscles of the androgen-sensitive SNB motoneurons contain very few estrogen receptors (fewer than 10% of muscle fibers express estrogen receptor α; Rudolph and Sengelaub, 2013), with very low binding affinity for estradiol (Dubé et al., 1976). The minimal expression of estrogen receptor in the SNB neuromuscular system readily explains the lack of any protective effects following treatment with estradiol (Fargo and Sengelaub, 2007; Verhovshek et al., 2010). In contrast, the quadriceps muscles contain estrogen receptors (Moncharmont and Parikh, 1983) with a binding affinity for estradiol that is over seven times higher than that seen in the SNB target muscles (Dubé et al., 1976). It is thus likely that the muscle as the site of action for the neuroprotective effect of estradiol in quadriceps motoneurons.

Support for the target muscle as the site of androgenic or estrogenic action comes from a variety of sources (Foecking et al., 2015). For example, motoneuron dendritic morphology is regulated by androgens acting at the target musculature in both the SNB (Rand and Breedlove, 1995) and quadriceps systems (in rats transgenically modified to overexpress androgen receptor exclusively in skeletal muscle, Huguenard et al., 2011). Androgen action at the target musculature has also been shown to regulate BDNF expression in motoneurons (Verhovshek and Sengelaub, 2013), and estrogens act at the SNB target muscle during postnatal development to support dendritic growth (Nowacek and Sengelaub, 2006). Most relevant to the current study, preliminary data from our lab show that following the selective death of motoneurons induced by saporin injection, induced dendritic atrophy in surviving

neighboring motoneurons was prevented by treatment with androgens or estrogens that was restricted locally to the target muscle (Cai et al., 2013). Together, these studies suggest a testable hypothesis that the target musculature is a critical site of action for the neuroprotective effects of gonadal hormones.

Conclusions

Treatment with androgens (Fargo et al., 2009a; Foecking et al., 2015) or estrogens (Henderson and Reynolds, 2002; Woolley and Cohen, 2002) promotes a wide array of neuroprotective and neurotherapeutic effects. The present results indicate that androgens and estrogens are also capable of attenuating secondary atrophy in surviving neighboring motoneurons, and that this neuroprotection requires action through steroid hormone receptors. Understanding the molecular mechanisms by which steroid hormones act on injured neurons is likely to provide important insights for developing therapeutic interventions for nervous system trauma and neurodegenerative diseases.

Acknowledgments

We thank Kellie Coons and Megan Downey for their assistance in data collection, and Dr. Cara Wellman and our anonymous reviewers for helpful comments on the manuscript. This work was supported by NIH-NINDS NS047264 to D.R.S.

References

Ahlbom E, Prins GS, Ceccatelli S. Testosterone protects cerebellar granule cells from oxidative stress-induced cell death through a receptor mediated mechanism. Brain Res. 2001; 892:255–262. [PubMed: 11172772]

Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB rnRNA in regenerating rat femoral motoneurons. Eur J Neurosci. 2000; 12:4381–4390. [PubMed: 11122348]

Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev. 2001; 81:1269–1304. [PubMed: 11427696]

Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci. 2007; 25:3039–3046. [PubMed: 17561817]

Bisby MA, Tetzlaff W. Changes in cytoskeletal protein synthesis following axon injury and during regeneration. Mol Neurobiol. 1992; 6:107–123. [PubMed: 1476674]

Breedlove SM, Arnold AP. Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. Science. 1980; 210:564–566. [PubMed: 7423210]

Breedlove SM, Arnold AP. Sex differences in the pattern of steroid accumulation by motoneurons of the rat lumbar spinal cord. J Comp Neurol. 1983; 215:211–216. [PubMed: 6853774]

Brushart TM, Seiler WA. Selective reinnervation of distal motor stumps by peripheral axons. Exp Neurol. 1987; 97:290–300.

Burke KA, Kuwajima M, Sengelaub DR. Aromatase inhibition reduces dendritic growth in a sexually dimorphic rat spinal nucleus. J Neurobiol. 1999; 38:301–312. [PubMed: 10022574]

Burke KA, Widows MR, Sengelaub DR. Synergistic effects of testosterone metabolites on the development of motoneuron morphology in a sexually dimorphic rat spinal nucleus. J Neurobiol. 1997; 33:1–10. [PubMed: 9212065]

Byers JS, Huguenard A, Kuruppu D, Liu N-K, Xu X-M, Sengelaub DR. Neuroprotective effects of testosterone on motoneuron and muscle morphology following spinal cord injury. J Comp Neurol. 2012; 520:2683–2696. [PubMed: 22314886]

Cai, Y., Chung, M., Mnayarji, J., Sengelaub, DR. Neuroprotection with gonadal steroids following partial motoneuron depletion: dependence on steroid receptor activation and hormone action at the target musculature. Society for Neuroscience Abstracts Viewer/Itinerary Planner; 2013. Program No. 467.12

Chang C, Kokontis J, Liao ST. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science. 1988a; 240:324–326. [PubMed: 3353726]

Chang C, Kokontis J, Liao ST. Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA. 1988b; 85:7211–7215. [PubMed: 3174628]

Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: Deciphering selective motor neuron death in ALS. Nat Rev Neurosci. 2001; 2:806–819. [PubMed: 11715057]

Dubé JY, Lesage R, Tremblay RR. Androgen and estrogen binding in rat skeletal and perineal muscles. Can J Biochem. 1976; 54:50–55. [PubMed: 175903]

Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, Roy RR. Plasticity of the spinal neural circuitry after injury. Annu Rev Neurosci. 2004; 27:145–167. [PubMed: 15217329]

Fargo KN, Foster AM, Harty MW, Sengelaub DR. Estrogen alters excitability but not morphology of a sexually dimorphic neuromuscular system in adult rats. J Neurobiol. 2003; 56:66–77. [PubMed: 12767033]

Fargo KN, Sengelaub DR. Testosterone manipulation protects motoneurons from dendritic atrophy after contralateral motoneuron depletion. J Comp Neurol. 2004a; 469:96–106. [PubMed: 14689475]

Fargo KN, Sengelaub DR. Exogenous testosterone prevents motoneuron atrophy induced by contralateral motoneuron depletion. J Neurobiol. 2004b; 60:348–359. [PubMed: 15281072]

Fargo KN, Sengelaub DR. Androgenic, but not estrogenic, protection of motoneurons from somal and dendritic atrophy induced by the death of neighboring motoneurons. Dev Neurobiol. 2007; 67:1094–1106. [PubMed: 17565709]

Fargo KN, Foecking EM, Jones KJ, Sengelaub DR. Neuroprotective actions of androgens on motoneurons. Front Neuroendocrinol. 2009a; 30:130–141. [PubMed: 19393684]

Fargo KN, Foster AM, Sengelaub DR. Neuroprotective effect of testosterone treatment on motoneuron recruitment following the death of nearby motoneurons. Dev Neurobiol. 2009b; 69:825–835. [PubMed: 19658088]

Foecking, EM., Fargo, KN., Brown, TJ., Sengelaub, DR., Jones, KJ. Gonadal steroids in regeneration and repair of neuromuscular systems. In: So, KF., Xu, X-M., editors. Neural Regeneration. Elsevier; London: 2015. p. 129-152.

Forger NG, Breedlove SM. Seasonal variation in mammalian striated muscle mass and motoneuron morphology. J Neurobiol. 1987; 18:155–165. [PubMed: 3572390]

Frye CA. The role of neurosteroids and non-genomic effects of progestins and androgens in mediating sexual receptivity of rodents. Brain Res Rev. 2001; 37:201–222. [PubMed: 11744087]

Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. Prog Neurobiol. 2000; 63:29–60.

Garcia-Segura LM, Veiga S, Sierra A, Melcangi RC, Azcoitia I. Aromatase: a neuroprotective enzyme. Prog Neurobiol. 2003; 71:31–41. [PubMed: 14611865]

Garrett JE, Wellman CL. Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence. Neuroscience. 2009; 162:195–207. [PubMed: 19401219]

Goldstein LA, Kurz EM, Kalkbrenner AE, Sengelaub DR. Changes in dendritic morphology of rat spinal motoneurons during development and after unilateral target deletion. Dev Brain Res. 1993; 73:151–163. [PubMed: 8353928]

Goldstein LA, Kurz EM, Sengelaub DR. Androgen regulation of dendritic growth and retraction in the development of a sexually dimorphic spinal nucleus. J Neurosci. 1990; 10:935–946. [PubMed: 2319307]

Goldstein LA, Sengelaub DR. Motoneuron morphology in the dorsolateral nucleus of the rat spinal cord: normal development and androgenic regulation. J Comp Neurol. 1993; 338:588–600. [PubMed: 7510730]

Goldstein LA, Sengelaub DR. Differential effects of dihydrotestosterone and estrogen on the development of motoneuron morphology in a sexually dimorphic rat spinal nucleus. J Neurobiol. 1994; 25:878–892. [PubMed: 8089663]

Hammond J, Le Q, Goodyer C, Gelfand M, Trifiro M, LeBlanc A. Testosterone-mediated neuroprotection through the androgen receptor in human primary neurons. J Neurochem. 2001; 77:1319–1326. [PubMed: 11389183]

Hebbeler SL, Sengelaub DR. Development of a sexually dimorphic neuromuscular system in male rats after spinal transection: morphologic changes and implications for estrogen sites of action. J Comp Neurol. 2003; 467:80–96. [PubMed: 14574681]

Hebbeler SL, Verhovshek T, Sengelaub DR. Ovariectomy attenuates dendritic growth in hormone-sensitive spinal motoneurons. J Neurobiol. 2001; 48:301–314. [PubMed: 11500843]

Hebbeler SL, Verhovshek T, Sengelaub DR. N-methyl-D-aspartate receptor blockade inhibits estrogenic support of dendritic growth in a sexually dimorphic rat spinal nucleus. J Comp Neurol. 2002; 451:142–152. [PubMed: 12209833]

Hebel, R., Stromberg, MW. Anatomy and Embryology of the Laboratory Rat. BioMed Verlag; Federal Republic of Germany: 1986.

Henderson, VW., Reynolds, DW. Protective effects of estrogen on aging and damaged neural systems. In: Pfaff, DW.Arnold, AP.Etgen, AM.Fahrbach, SE., Rubin, RT., editors. Hormones, Brain and Behavior. San Diego: Academic Press; 2002. p. 821-837.

Hoang TX, Nieto J, Tillakaratne NJK, Havton LA. Autonomic and motor neuron death is progressive and parallel in a lumbosacral ventral root avulsion model of cauda equina injury. J Comp Neurol. 2003; 467:477–486. [PubMed: 14624482]

Hoffman GE, Merchenthaler I, Zup SL. Neuroprotection by ovarian hormones in animal models of neurological disease. Endocrine. 2006; 29:217–231. [PubMed: 16785598]

Huguenard A, Fernando SM, Monks DA, Sengelaub DR. Overexpression of androgen receptors in target musculature confers androgen sensitivity to motoneuron dendrites. Endocrinol. 2011; 152:639–650.

Huppenbauer CB, Tanzer L, DonCarlos LL, Jones KJ. Gonadal steroid attenuation of developing hamster facial motoneuron loss by axotomy: equal efficacy of testosterone, dihydrotestosterone, and 17-beta estradiol. J Neurosci. 2005; 25:4004–4013. [PubMed: 15843602]

Jones KJ, Brown TJ, Damaser M. Neuroprotective effects of gonadal steroids on regenerating peripheral motoneurons. Brain Res Rev. 2001; 37:372–382. [PubMed: 11744101]

Kalb RG. Regulation of motor neuron dendrite growth by NMDA receptor activation. Development. 1994; 120:3063–3071. [PubMed: 7720552]

Keefer DA, Stumpf WE, Sar M. Estrogen-topographical localization of estrogen-concentrating cells in the rat spinal cord following ^{3}H-estradiol administration. Proc Soc Exp Biol Med. 1973; 143:414–417. [PubMed: 4350785]

Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. Neurol. 1968; 18:671–680.

Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: New roles for the synaptic stripper. Neuron. 2013; 77:10–18. [PubMed: 23312512]

Kiley, BJ., Sengelaub, DR. Neuroprotection with androgens following partial motoneuron depletion: A role for microglia. Society for Neuroscience Abstracts Viewer/Itinerary Planner; 2015. Program No. 689.18

Kochakian CD. Definition of androgens and protein anabolic steroids. Pharmac Therap B. 1975; 1:149–177.

Kujawa KA, Emeric E, Jones KJ. Testosterone differentially regulates the regenerative properties of injured hamster facial motoneurons. J Neurosci. 1991; 11:3898–3906. [PubMed: 1744696]

Kujawa KA, Jones KJ. Testosterone-induced acceleration of recovery from facial paralysis in male hamsters: temporal requirements of hormone exposure. Physiol Behav. 1990; 48:765–768. [PubMed: 2082378]

Kujawa KA, Kinderman NB, Jones KJ. Testosterone-induced acceleration of recovery from facial paralysis following crush axotomy of the facial nerve in male hamsters. Exp Neurol. 1989; 105:80–85. [PubMed: 2744130]

Kumar R, Thompson EB. The structure of the nuclear hormone receptors. Steroids. 1999; 64:310–319. [PubMed: 10406480]

Kurz EM, Brewer RG, Sengelaub DR. Hormonally mediated plasticity of motoneuron morphology in the adult rat spinal cord: a cholera toxin-HRP study. J Neurobiol. 1991; 22:976–988. [PubMed: 1795161]

Kurz EM, Sengelaub DR, Arnold AP. Androgens regulate the dendritic length of mammalian motoneurons in adulthood. Science. 1986; 232:395–398. [PubMed: 3961488]

Lange CA, Gioeli D, Hammes SR, Marker PC. Integration of rapid signaling events with steroid hormone receptor action in breast and prostate cancer. Annu Rev Physiol. 2007; 69:171–199. [PubMed: 17037979]

Leslie M, Forger NG, Breedlove SM. Does androgen affect axonal transport of cholera toxin HRP in spinal motoneurons? Neurosci Lett. 1991; 126:199–202. [PubMed: 1922934]

Little CM, Coons KD, Sengelaub DR. Neuroprotective effects of testosterone on the morphology and function of somatic motoneurons following the death of neighboring motoneurons. J Comp Neurol. 2009; 512:359–372. [PubMed: 19003970]

Liu NK, Byers JS, Lam T, Lu Q-B, Sengelaub DR, Xu X-M. Inhibition of cPLA2 has neuroprotective effects on motoneuron and muscle atrophy following spinal cord injury. J Neurotrauma. 2014a; doi: 10.1089/neu.2014.3690

Liu N-K, Deng L-X, Zhang YP, Lu Q-B, Wang X-F, Hu J-G, Oakes E, Shields CB, Xu X-M. Cytosolic phospholipase A2 protein as a novel therapeutic target for spinal cord injury. Ann Neurol. 2014b; 75:644–58. [PubMed: 24623140]

Lumbroso S, Sandillon F, Georget V, Lobaccaro JM, Brinkmann AO, Privat A, Sultan C. Immunohistochemical localization and immunoblotting of androgen receptor in spinal neurons of male and female rats. Eur J Endocrinol. 1996; 134:626–632. [PubMed: 8664984]

Matsuura T, Ogata A, Demura T, Moriwaka F, Tashiro K, Koyanagi T. Identification of androgen receptor in the rat spinal motoneurons. Immunohistochemical and immunoblotting analyses with monoclonal antibody. Neurosci Lett. 1993; 158:5–8. [PubMed: 8233073]

Mesulam, MM. Tracing Neural Connections with Horseradish Peroxidase. Chichester: Wiley; 1982.

Molander C, Grant G. Spinal cord projections from hindlimb muscle nerves in the rat studied by transganglionic transport of horseradish peroxidase, wheat germ agglutinin conjugated horseradish peroxidase, or horseradish peroxidase with dimethylsulfoxide. J Comp Neurol. 1987; 260:246–255. [PubMed: 3038969]

Moncharmont B, Parikh I. Binding of monoclonal antibodies to the nuclear estrogen receptor in intact nuclei. Biochem Biophys Res Commun. 1983; 114:107–112. [PubMed: 6882416]

Monks DA, Kopachik W, Breedlove SM, Jordan CL. Anabolic responsiveness of skeletal muscles correlates with androgen receptor protein but not mRNA. Can J Physiol Pharmacol. 2006; 84:272–277.

Morrell JI, Wolinsky TD, Krieger MS, Pfaff DW. Autoradiographic identification of estradiol-concentrating cells in the spinal cord of the female rat. Exp Brain Res. 1982; 45:144–150. [PubMed: 7056321]

Moschilla G, Song S, Chakera T. Post-traumatic lumbar nerve root avulsion. Austral Radiol. 2001; 45:281–284.

Nicolopoulos-Stournaras S, Iles JF. Motor neuron columns in the lumbar spinal cord of the rat. J Comp Neurol. 1983; 217:75–85. [PubMed: 6875053]

Nowacek AS, Sengelaub DR. Estrogenic support of motoneuron dendritic growth via the neuromuscular periphery in a sexually dimorphic motor system. J Neurobiol. 2006; 66:962–976. [PubMed: 16779828]

O'Hanlon GM, Lowrie MB. Nerve injury in adult rats causes abnormalities in the motoneuron dendritic field that differ from those seen following neonatal nerve injury. Exp Brain Res. 1995; 103:243–250. [PubMed: 7789431]

Overpeck JG, Colson SH, Hohmann JR, Applestine MS, Reilly JF. Concentrations of circulating steroids in normal prepubertal and adult male and female humans, chimpanzees, rhesus monkeys, rats, mice, and hamsters: A literature survey. J Toxicol Environ Health. 1978; 4:785–803. [PubMed: 104044]

Osborne MC, Verhovshek T, Sengelaub DR. Androgen regulates trkB expression in spinal motoneurons. J Neurosci Res. 2007; 85:303–309. [PubMed: 17131419]

Piccone CM, Brazeau GA, McCormick KM. Effect of oestrogen on myofibre size and myosin expression in growing rats. Exp Physiol. 2005; 90:87–93. [PubMed: 15466459]

Pike CJ. Testosterone attenuates ß-amyloid toxicity in cultured hippocampal neurons. Brain Res. 2001; 919:160–165. [PubMed: 11689174]

Porter KL, Shetty G, Shuttlesworth GA, Weng CC, Huhtaniemi I, Pakarinen P, Meistrich ML. Estrogen enhances recovery from radiation-induced spermatogonial arrest in rat testes. J Androl. 2009; 30:440–51. [PubMed: 19136390]

Ramsden M, Shin TM, Pike CJ. Androgens modulate neuronal vulnerability to kainate lesion. Neuroscience. 2003; 122:573–578. [PubMed: 14622899]

Rand MN, Breedlove SM. Androgen alters the dendritic arbors of SNB motoneurons by acting upon their target muscles. J Neurosci. 1995; 15:4408–4416. [PubMed: 7540674]

Rudolph LM, Sengelaub DR. Critical period for estrogen-dependent motoneuron dendrite growth is coincident with ERα expression in target musculature. Dev Neurobiol. 2013; 73:72–84. [PubMed: 22678724]

Sen A, Prizant H, Hammes SR. Understanding extranuclear (nongenomic) androgen signaling: what a frog oocyte can tell us about human biology. Steroids. 2011; 76:822–828. [PubMed: 21354434]

Sengelaub, DR., Osborne, MC., Little, CM., Huyck, KD., Verhovshek, T. Neuroprotective effects of testosterone in two models of spinal motoneuron injury. Society for Neuroscience Abstracts Viewer/Itinerary Planner; 2006. Program No. 683.12

Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. J Comp Neurol. 1990; 294:76–95. [PubMed: 2324335]

Sfikakis A, Spyraki C, Sitaras N, Varonos D. Implication of the estrous cycle on conditioned avoidance behavior in the rat. Physiol Behav. 1978; 21:441–446. [PubMed: 570277]

Smith, ER., Damassa, DA., Davidson, JM. Hormone Administration: Peripheral and Intracranial Implants. In: Meyer, RD., editor. Methods in Psychobiology. New York: Academic Press; 1977. p. 259-279.

Stirpe F, Barbieri L, Battelli MG, Soria M, Lappi DA. Ribosome-inactivating proteins from plants: present status and future prospects. Bio/Technology. 1992; 10:405–412. [PubMed: 1368484]

Stirpe F, Gasperi-Campani A, Barbieri L, Falasca A, Abbondanza A, Stevens WA. Ribosome-inactivating proteins from the seeds of *Saponaria officinalis* L. (soapwort), of *Agrostemma githago* L. (corn cockle) and from *Asparagus officinalis* L. (asparagus), and from the latex of *Hura crepitans* L. (sandbox tree). Biochem J. 1983; 216:617–625. [PubMed: 6667259]

Sumner BEH, Watson WE. Retraction and expansion of the dendritic tree of motor neurons of adult rats induced in vivo. Nature. 1971; 233:273–275. [PubMed: 4938371]

Tanzer L, Jones KJ. Gonadal steroid regulation of hamster facial nerve regeneration: effects of dihydrotestosterone and estradiol. Exp Neurol. 1997; 146:258–264. [PubMed: 9225759]

Thomas P. Rapid steroid hormone actions initiated at the cell surface and the receptors that mediate them with an emphasis on recent progress in fish models. Gen Comp Endocrinol. 2012; 175:367–383. [PubMed: 22154643]

Tremblay RR, Dubé JY, Ho-Kim MA, Lesage R. Determination of rat muscles androgen-receptor complexes with methyltrienolone. Steroids. 1977; 29:185–195. [PubMed: 841621]

Titmus MJ, Faber DS. Axotomy-induced alterations in the electrophysiological characteristics of neurons. Prog Neurobiol. 1990; 35:1–51. [PubMed: 2217820]

Verhovshek T, Sengelaub DR. Trophic effects of BDNF blockade in an androgen-sensitive neuromuscular system. Endocrinol. 2010; 151:5337–5348.

Verhovshek T, Sengelaub DR. Androgen action at the target musculature regulates brain-derived neurotrophic factor protein in the spinal nucleus of the bulbocavernosus. Dev Neurobiol. 2013; 73:587–598. [PubMed: 23512738]

Williams SJ, Papka RE. Estrogen receptor-immunoreactive neurons are present in the female rat lumbosacral spinal cord. J Neurosci Res. 1996; 46:492–501. [PubMed: 8950709]

Wilson RE, Coons KD, Sengelaub DR. Neuroprotective effects of testosterone on dendritic morphology following partial motoneuron depletion: Efficacy in female rats. Neurosci Lett. 2009; 465:123–127. [PubMed: 19735695]

Woolley, CS., Cohen, RS. Sex steroids and neuronal growth in adulthood. In: Pfaff, DW.Arnold, AP.Etgen, AM.Fahrbach, SE., Rubin, RT., editors. Hormones, Brain and Behavior. San Diego: Academic Press; 2002. p. 717-777.

Yang L, Verhovshek T, Sengelaub DR. BDNF and androgen interact in the maintenance of dendritic morphology in a sexually dimorphic rat spinal nucleus. Endocrinol. 2004; 145:161–168.

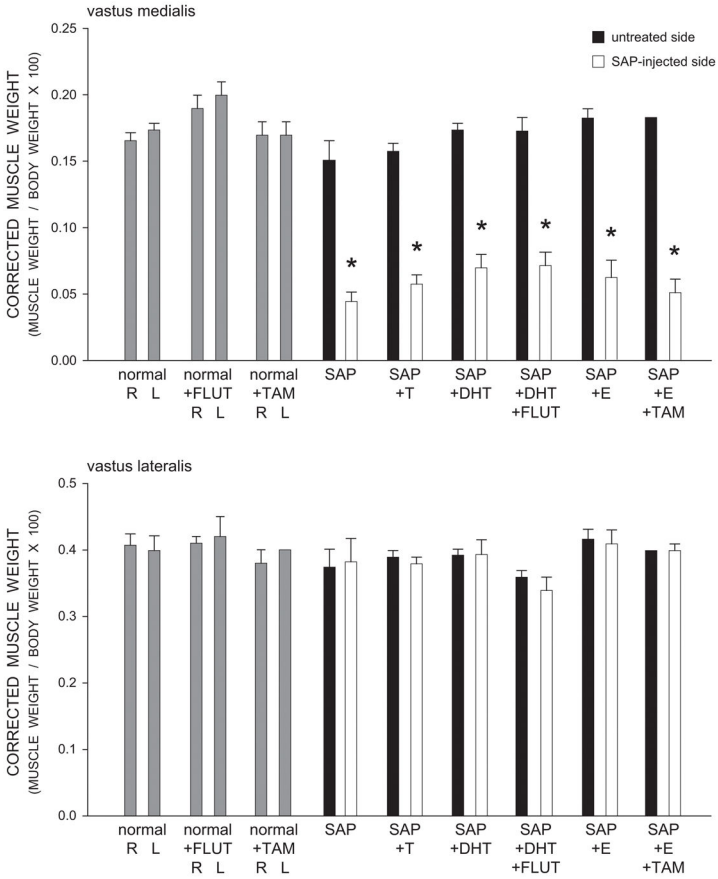

Figure 1.

Weights of the vastus medialis (top) and vastus lateralis (bottom) muscles corrected by body weight in normal animals and saporin-injected animals that were either untreated (SAP), or treated with testosterone (SAP+T), dihydrotestosterone (SAP+DHT), dihydrotestosterone and flutamide (SAP+DHT+FLUT), estradiol (SAP+E), or estradiol and tamoxifen (SAP+E +TAM). Gray bars represent weights from the right (R) and left (L) sides in normal animals, either untreated or treated with flutamide (normal+FLUT) or tamoxifen (normal+TAM). Black bars represent weights from the untreated contralateral (right) side of the quadriceps muscle, and white bars represent weights from the saporin injected (left) side of the quadriceps muscle of saporin-injected animals. Saporin injection reduced the weight of the vastus medialis muscle but had no effect on the adjacent vastus lateralis muscle. Treatment with hormones or receptor antagonists had no effect on muscle weight. Bar heights represent means ± SEM. * indicates significantly different from normal animals.

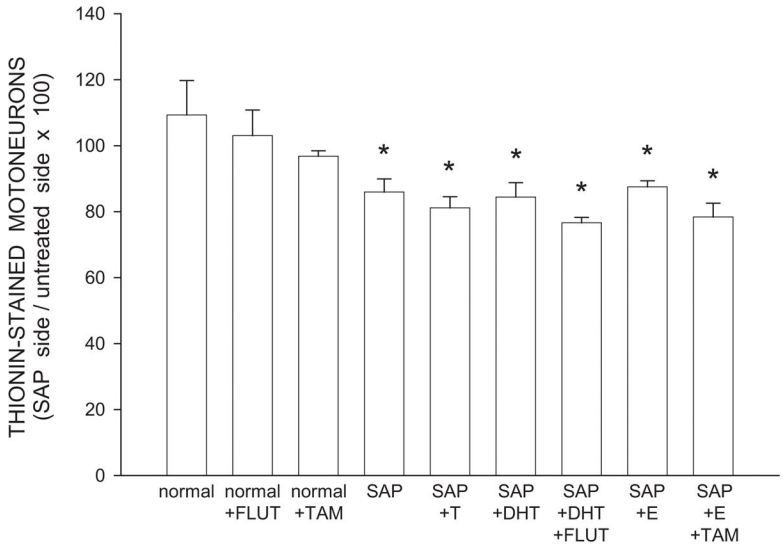

Figure 2.

Numbers of thionin-stained quadriceps motoneurons in normal animals, either untreated or treated with flutamide (normal+FLUT) or tamoxifen (normal+TAM), and saporin-injected animals that were either untreated (SAP), or treated with testosterone (SAP+T), dihydrotestosterone (SAP+DHT), dihydrotestosterone and flutamide (SAP+DHT+FLUT), estradiol (SAP+E), or estradiol and tamoxifen (SAP+E+TAM), expressed as a ratio of motoneuron number ipsilateral to the saporin-injected muscle relative to that on the untreated side. Saporin killed approximately 25% of the ipsilateral quadriceps motoneurons, regardless of subsequent treatment. Treatment of otherwise normal animals with receptor antagonists had no effect on motoneuron number. Bar heights represent means ± SEM. * indicates significantly different from normal animals.

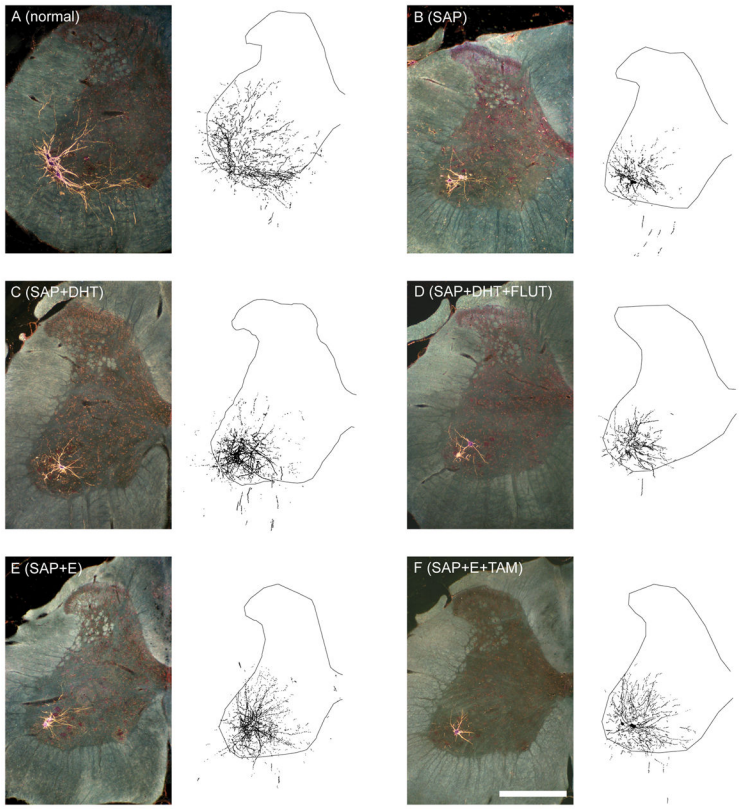

Figure 3.

Darkfield digital micrographs of transverse hemisections through the lumbar spinal cords and computer-generated reconstructions BHRP-labeled somata and processes of a normal animal (A), and saporin-injected animals left untreated (B), or treated with dihydrotestosterone (C), dihydrotestosterone and flutamide (D), estradiol (E), or estradiol and tamoxifen (F) after BHRP injection into the left vastus lateralis muscle. 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.

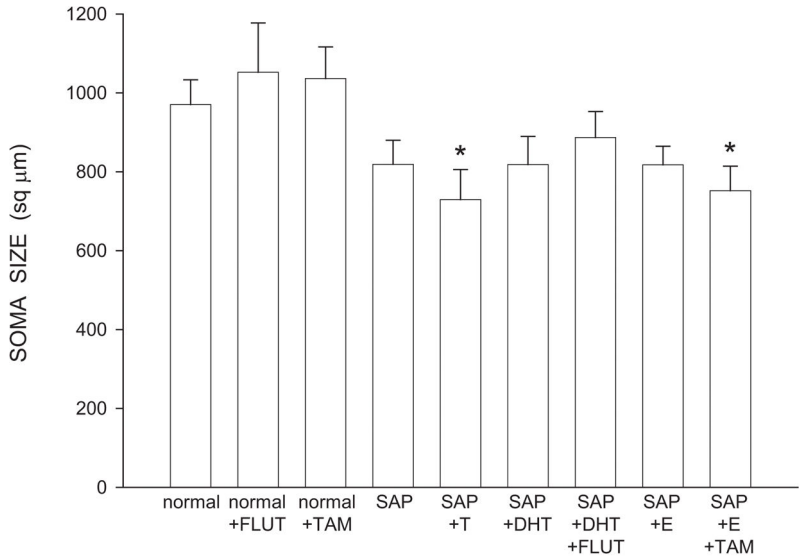

Figure 4.

Cross-sectional soma areas of quadriceps motoneurons in normal animals, either untreated or treated with flutamide (normal+FLUT) or tamoxifen (normal+TAM), and saporin-injected animals that were either untreated (SAP), or treated with testosterone (SAP+T), dihydrotestosterone (SAP+DHT), dihydrotestosterone and flutamide (SAP+DHT+FLUT), estradiol (SAP+E), or estradiol and tamoxifen (SAP+E+TAM). Following saporin-induced motoneuron death, the average soma areas of surviving neighboring motoneurons decreased by approximately 17%, regardless of hormone treatment. Treatment of otherwise normal animals with receptor antagonists had no effect on soma area. Bar heights represent means ± SEM. * indicates significantly different from normal animals.

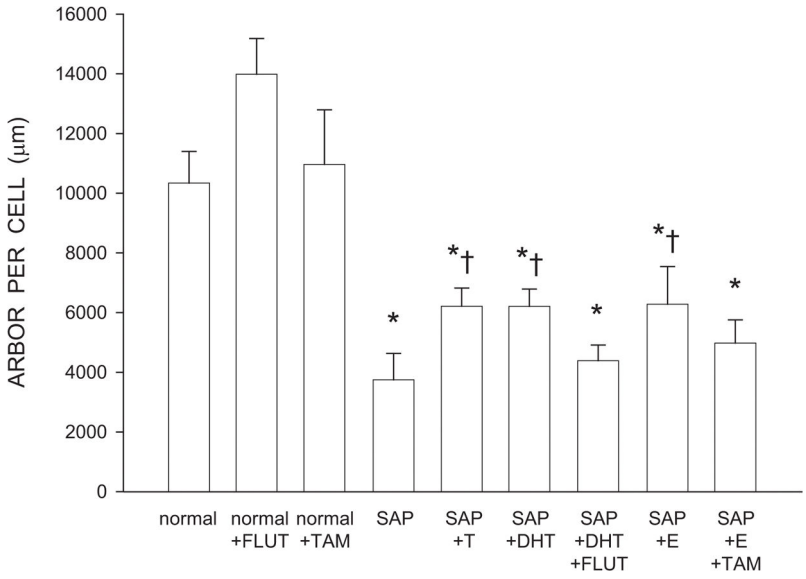

Figure 5.

Dendritic lengths of quadriceps motoneurons in normal animals, either untreated or treated with flutamide (normal+FLUT) or tamoxifen (normal+TAM), and saporin-injected animals that were either untreated (SAP), or treated with testosterone (SAP+T), dihydrotestosterone (SAP+DHT), dihydrotestosterone and flutamide (SAP+DHT+FLUT), estradiol (SAP+E), or estradiol and tamoxifen (SAP+E+TAM). Following saporin-induced motoneuron death, surviving neighboring motoneurons lost almost 64% of their dendritic length. Treatment with testosterone, dihydrotestosterone, or estradiol attenuated this dendritic atrophy, but blockade of androgen or estrogen receptors prevented this attenuation. Treatment of otherwise normal animals with receptor antagonists had no effect on dendritic length. Bar heights represent means ± SEM. * indicates significantly different from normal animals. † indicates significantly different from untreated saporin-injected animals.

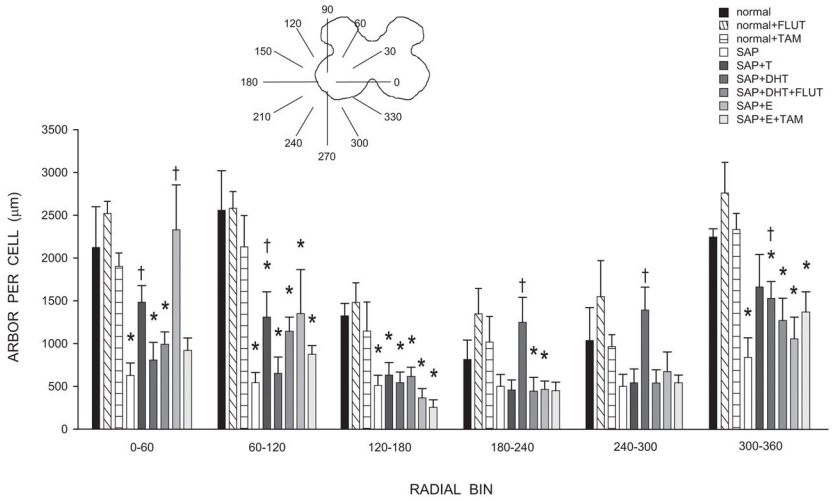

Figure 6.

Inset: Drawing of spinal gray matter divided into radial sectors for measure of quadriceps motoneuron dendritic distribution. Length per radial bin of quadriceps dendrites in normal animals, either untreated (black bars) or treated with flutamide (normal+FLUT, diagonal hatched bars) or tamoxifen (normal+TAM, horizontal hatched bars), and saporin-injected animals that were either untreated (SAP, white bars), or treated with testosterone (SAP+T, darkest gray bars), dihydrotestosterone (SAP+DHT, dark gray bars), dihydrotestosterone and flutamide (SAP+DHT+FLUT, gray bars), estradiol (SAP+E, light gray bars), or estradiol and tamoxifen (SAP+E+TAM, lightest gray bars). For graphic purposes, dendritic length measures have been collapsed into 6 bins of 60° each. Quadriceps motoneuron dendritic arbors display a non-uniform distribution, with the majority of the arbor located between 300° and 120°. Following saporin-induced motoneuron death, surviving neighboring motoneurons had reduced dendritic length in every radial bin. Treatment with testosterone, dihydrotestosterone, and estradiol attenuated this reduction, but blockade of androgen or estrogen receptors prevented this attenuation. Treatment of otherwise normal animals with receptor antagonists had no effect on dendritic distribution. Bar heights represent means ± SEM. * indicates significantly different from normal animals. † indicates significantly different from untreated saporin-injected animals.

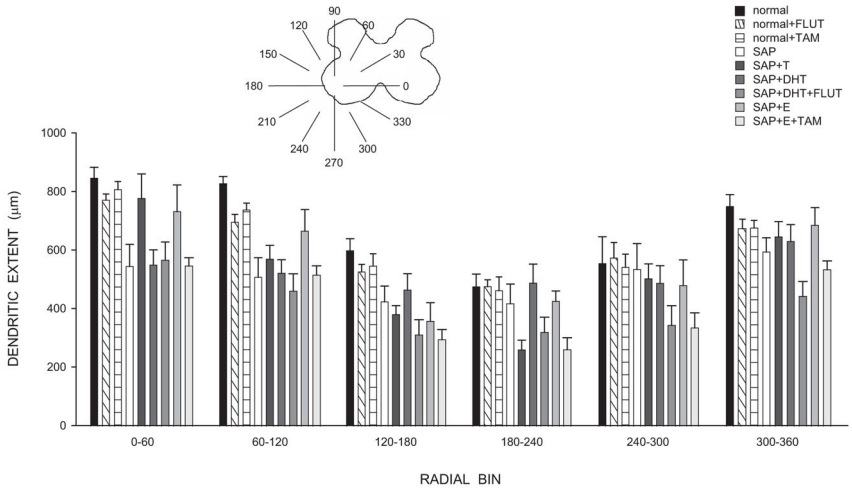

Figure 7.

Inset: Drawing of spinal gray matter divided into radial sectors for measure of quadriceps motoneuron radial dendritic extent. Radial extents of quadriceps dendrites in normal animals, either untreated (black bars) or treated with flutamide (normal+FLUT, diagonal hatched bars) or tamoxifen (normal+TAM, horizontal hatched bars), and saporin-injected animals that were either untreated (SAP, white bars), or treated with testosterone (SAP+T, darkest gray bars), dihydrotestosterone (SAP+DHT, dark gray bars), dihydrotestosterone and flutamide (SAP+DHT+FLUT, gray bars), estradiol (SAP+E, light gray bars), or estradiol and tamoxifen (SAP+E+TAM, lightest gray bars). For graphic purposes, dendritic extent measures have been collapsed into 6 bins of 60° each. Extent measures did not differ across groups. Bar heights represent means ± SEM.