Published in final edited form as:

Neurosci Lett. 2009 November 13; 465(2): 123–127. doi:10.1016/j.neulet.2009.09.007.

Neuroprotective effects of testosterone on dendritic morphology following partial motoneuron depletion: Efficacy in female rats

Randall E. Wilson, Kellie D. Coons, and Dale R. Sengelaub*

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

Abstract

Motoneuron loss is a significant medical problem, capable of causing severe movement disorders and even death. We have previously demonstrated that partial depletion of motoneurons induces dendritic atrophy in remaining motoneurons, with a concomitant reduction in motor activation. Treatment of male rats with testosterone attenuates the regressive changes following partial motoneuron depletion. To test whether testosterone has similar effects in females, we examined potential neuroprotective effects in motoneurons innervating muscles of the quadriceps of female rats. Motoneurons were selectively killed by intramuscular injection of cholera toxin-conjugated saporin. Simultaneously, some saporin-injected rats were given implants containing testosterone or left untreated. Four weeks later, surviving motoneurons were labeled with cholera toxin-conjugated HRP, and dendritic arbors were reconstructed in 3 dimensions. Compared to normal females, partial motoneuron depletion resulted in decreased dendritic length in remaining quadriceps motoneurons, and this atrophy was greatly attenuated by testosterone treatment. These findings suggest that testosterone has neuroprotective effects on morphology in both males and females, further supporting a role for testosterone as a neurotherapeutic agent in the injured nervous system.

Keywords

steroids; neuroprotection; morphology; dendrites

Motoneuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophies (e.g., spinal and bulbar muscular atrophy) are characterized by progressive loss of motoneurons [3,17]. Similarly, damage to spinal nerves resulting in laceration and avulsion of spinal roots can lead to the death of motoneurons and preganglionic autonomic neurons in the spinal cord, resulting in autonomic and motor dysfunction [16]. However, the death of motoneurons is not the only outcome, and after such insults surviving motoneurons undergo dendritic retraction and atrophy [e.g., 2,28] as well as functional and biochemical changes [e.g., 1,33]. We have been examining the effects of motoneuron loss on the structure and function of surviving motoneurons using a rat model of motoneuron death. Partial depletion of motoneurons results in substantial somal and dendritic atrophy [10–12,23] and reduced excitability [9,23] in the remaining motoneurons.

*Correspondence to: Dale R. Sengelaub, PhD, Psychology Building, Indiana University, 1101 East 10th Street, Bloomington, Indiana 47405, Phone: (812) 855-9149, Fax: (812) 855-4691, sengelau@indiana.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Androgens exhibit a wide array of neuroprotective and neurotherapeutic effects in motoneurons [8]. Our previous studies have demonstrated that testosterone is neuroprotective following motoneuron loss. Treatment with exogenous testosterone protects surviving motoneurons from dendritic atrophy and the concomitant attenuated excitability induced by motoneuron loss [9–12,23]. Furthermore, neuroprotection with testosterone occurs in multiple motor populations [10,23], is dose-dependent [6], and has a therapeutic window that extends long after the initial injury [5].

However, to date all of our work has used male rats, and given the multiple sex differences in endocrinological, neural, and somatic organization, it is possible that the androgenic effects we observed in motoneurons after induced motoneuron depletion may be restricted to males. Thus, in this experiment we tested whether androgen treatment would similarly attenuate dendritic atrophy after induced motoneuron depletion in females.

In rats, the quadriceps muscle is comprised of the vastus lateralis, the vastus medialis, the vastus intermedialis, and the rectus femoris. These muscles are innervated by motoneurons located in the lateral motor column in the L2 spinal segment [27]. 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 [27,30,31]. By selectively depleting motoneurons from this population, we can study the effects of that depletion on remaining, neighboring motoneurons, as well as any potential protective effects of androgen treatment.

Adult female Sprague Dawley rats (approximately 100 days old; Harlan) were maintained on a 12:12 hour light/dark cycle, with unlimited access to food and water. 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 μ l, 0.1%; Advanced Targeting Systems, Inc.). Cholera toxin-conjugated saporin is retrogradely transported from the site of injection and kills motoneurons innervating the injected musculature within 3–6 days [10].

Some of the female rats were not treated further (n = 4) while others were immediately implanted with subcutaneous Silastic capsules filled with testosterone (4-androsten-17 β -ol-3-one; Steraloids; 3.18 mm O.D., 1.57 mm I.D., 45 mm long; n = 6). Such implants produce plasma titers of testosterone in the normal physiological range for adult males [2.5 ng/ml; 32], and have previously been demonstrated to attenuate motoneuron atrophy and loss of function induced by the death of nearby motoneurons [10–12,23]. Animals in both saporin-injected groups were allowed to survive for four weeks following saporin injection, a length of time sufficient to observe saporin-induced effects on morphology [10–12,23]. An additional group of age-matched, untreated females served as normal controls (n = 8).

Four weeks after saporin injection, animals were re-anesthetized, and the left vastus lateralis muscle (ipsilateral to the saporin-injected vastus medialis muscle in saporin animals) was exposed and injected with horseradish peroxidase conjugated to the cholera toxin B subunit (BHRP; 2 μ l, 0.2%; List Biological, Inc.). BHRP labeling permits population-level quantitative analysis of motoneuron somal and dendritic morphologies [14]. Forty-eight hours after BHRP injection, a period that ensures optimal labeling of motoneurons [14], animals were weighed, overdosed with 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 lateralis and vastus medialis 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 and frozen-sectioned transversely at 40 μ m; 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 [26], mounted on gelatin-coated slides, and counterstained with thionin.

The appropriate area within the lateral motor column for motoneuron counts in the unreacted series was identified using our previously described method [23,30]. Briefly, for each animal the range of sections in which motoneurons labeled with BHRP were present in the reacted series was identified, and then motoneuron counts were performed 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 outlined by Coggeshall [4] as described previously (see [23] for details). Counts were made at 937.5X under brightfield illumination. Motoneurons are easily recognizable as large, darkly staining, multipolar cells. Cell counts for each animal were multiplied by four to correct 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.

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.

The size of quadriceps motoneuron somata was assessed in at least one set of alternate sections (160 μ m apart) by measuring the cross-sectional area of BHRP-filled motoneurons. Soma areas of an average of 19.9 motoneurons were measured for each animal using a video-based morphometry system (Stereo Investigator; MicroBrightField) at a final magnification of 780X. Soma areas within each animal were then averaged for statistical analysis.

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 in every third section (480 μ m apart) in three dimensions using a computer-based morphometry system (Neurolucida; MicroBrightField) at a final magnification of 250X. Average dendritic length per labeled motoneuron ("arbor per cell") 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 [21], but has been shown to be a sensitive and reliable measure of dendritic morphology after a variety of manipulations, including changes after the death of nearby motoneurons [10–12,23].

To assess potential redistributions of dendrites, for each animal a composite of the dendritic arbor and somata in all traced sections was created and divided into twelve radial bins (30°) in the transverse plane using a set of axes oriented around the geometric center of the collective labeled somata. The portion of each animal's dendritic arbor contained within each of the bins was then determined.

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 quadriceps motoneuron 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 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 the Indiana University 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 using an MDS 290 digital camera system (Eastman Kodak Company). Brightness and contrast of these images were adjusted in Adobe Photoshop.

Overall body weight was not affected; animals weighed an average of 289.57 ± 10.74 g (Mean $\pm$ SEM) at the end of treatment, and this did not differ between groups. However, muscle weights were affected by saporin injection (Fig. 1). In normal females, the weights of the right (0.47 ± 0.03 g) and left (0.46 ± 0.02 g) vastus medialis muscles were similar. While the weights of the uninjected (right) vastus medialis muscles were not affected [$F(2,15) = 0.63$, *ns*], unilateral injection of saporin into the left vastus medialis resulted in marked atrophy of the injected musculature across the saporin groups [an average 60.8% reduction in weight; $F(2,15) = 27.83$, $p < .05$].

Notably, the effect of saporin injection on quadriceps weight was specific to the injected muscle. In normal females, the weights of the right (1.03 ± 0.04 g) and left (1.05 ± 0.03 g) vastus lateralis muscles were similar. In the saporin groups, the weights of the vastus lateralis muscles on the untreated (right) side (1.18 ± 0.04 g, saporin-injected females; 1.19 ± 0.02 g, testosterone-treated saporin females) did not differ from those of normal females [$F(2,15) = 0.08$, *ns*]. Most importantly, the weights of the vastus lateralis muscles (1.20 ± 0.07 g, saporin-injected females; 1.17 ± 0.05 g, testosterone-treated saporin females) adjacent to the saporin-injected vastus medialis muscles also did not differ across groups [$F(2,15) = 0.01$, *ns*].

In normal females, the number of motoneurons within the identified quadriceps range did not differ between the left (484.0 ± 91.15) and right (489.1 ± 92.54) motor columns [paired t-test, $t(7) = -0.85.3$, *ns*]. 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 [$F(2,15) = 17.32$, $p < .05$]. Motoneuron numbers were reduced 24.5% in saporin-injected females, and treatment with testosterone did not prevent this reduction (20.3%); motoneuron counts in the saporin-injected groups did not differ (LSD, *ns*), and both differed from those of normal females (LSDs $< .05$).

Injection of BHRP into the left vastus lateralis successfully labeled ipsilateral quadriceps motoneurons in all groups (Fig. 2a). The dendritic arbor of quadriceps motoneurons was strictly unilateral, with extensive ramification along the ventrolateral edges of the gray matter and in the lateral funiculus, as well as throughout the ventral horn. An average of $30.44 (\pm 1.19)$ motoneurons per animal were labeled with BHRP, and this did not vary across groups [$F(2,15) = 1.36$, *ns*].

Following saporin-induced motoneuron death, no significant differences in soma size were observed in surviving nearby quadriceps motoneurons. Soma areas in saporin-injected animals ($918.92 \pm 118.53 \mu\text{m}^2$) and testosterone-treated saporin animals ($847.74 \pm 50.79 \mu\text{m}^2$) were not significantly different from those of normal females [$1013.62 \pm 78.68 \mu\text{m}^2$; $F(2,15) = 1.22$, *ns*].

Following saporin-induced motoneuron death, surviving nearby quadriceps motoneurons underwent marked dendritic atrophy. Dendritic length decreased by 62.6% [2734.33 ± 595.64

μm in saporin-injected animals compared to $7126.23 \pm 1030.13 \mu\text{m}$ for normal females, LSD, $p < .05$; overall test for the effect of group on arbor per cell $F(2,15) = 3.85$, $p < .05$; Fig. 2b]. Reductions in dendritic length occurred uniformly throughout the radial distribution, ranging from 69.7% (300° to 360°) to 54.6% (0° to 60°) in saporin-injected animals compared to normal females [$F(1,99) = 6.27$, $p < .05$]. Treatment with testosterone greatly attenuated induced dendritic atrophy: although dendritic lengths were reduced by 7.5% ($6592.52 \pm 1202.29 \mu\text{m}$) in testosterone-treated saporin animals compared to those of normal females, this difference was not significant (LSD, *ns*). This attenuation with testosterone treatment was present throughout the radial distribution [$F(1,121) = 0.437$, *ns*].

Radial dendritic extent did not differ across groups [$F(2,154) = 1.99$, *ns*]. Rostrocaudal dendritic extent also did not differ across groups [$F(2,15) = 1.80$, *ns*], spanning $3588.6 \pm 393.25 \mu\text{m}$ in normal females, $2600.00 \pm 528.14 \mu\text{m}$ in saporin-injected animals, and $3573.33 \pm 232.47 \mu\text{m}$ in testosterone-treated saporin animals.

In this experiment we tested whether the neuroprotective effects of testosterone in surviving motoneurons previously observed in males would be present in female rats. Partial motoneuron depletion resulted in decreased dendritic length in remaining quadriceps motoneurons. Importantly, testosterone treatment attenuated dendritic atrophy, indicating that the neuroprotective effects of testosterone we have previously reported in male rats appear to be present in female rats as well.

Saporin injection decreased the weight of the injected vastus medialis muscle, but had no effect on the weight of the adjacent uninjected vastus lateralis muscle, indicating that saporin treatments were both effective and confined to the targeted musculature. Treatment with testosterone did not prevent the reduction in muscle weight resulting from saporin injection: the weights of the vastus medialis muscles in the saporin-injected groups did not differ, being reduced by over 60%. Treatment with testosterone also had no effect on the weight of the non-saporin-injected vastus lateralis muscles, either those adjacent to the saporin-injected muscle or in the contralateral limb. Because the weight of the contralateral vastus lateralis muscles was not affected by testosterone 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 of testosterone by saporin. Although androgens are known to have protein anabolic effects on general skeletal muscle tissue [18], these effects are small compared to those observed in highly androgen-sensitive, sexually dimorphic muscles (e.g., the perineal muscles, [19,34]). Because the dendritic atrophy in remaining quadriceps motoneurons was attenuated by testosterone treatment while no effects were seen in target muscle weight, it is unlikely that any potential neurotrophic support from the musculature is dependent on muscle size alone.

Saporin injection into only one of the four muscles comprising the quadriceps resulted in the death of ipsilateral motoneurons, reducing the number in the quadriceps pool by approximately 22% across both saporin-injected groups. 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. Thus, the beneficial effects of steroid treatment on the morphology of nearby surviving motoneurons cannot be attributed to a hormone-mediated attenuation of the ability of saporin to kill motoneurons.

Saporin-induced motoneuron death also resulted in a pronounced dendritic atrophy in surviving nearby quadriceps motoneurons. Dendritic length of quadriceps motoneurons was decreased by almost 63% in saporin-injected females, a magnitude of atrophy almost identical to that seen in males (64%, [23]). This reduction was not restricted to any specific portion of the dendritic distribution, suggesting that the effect was the result of a general effect on the

quadriceps motoneurons rather than a loss of a specific afferent population [15]. Also similar to our previous reports for quadriceps motoneurons in males, atrophy of quadriceps motoneuron dendrites was attenuated in testosterone-treated saporin females, but in the present case to a greater degree. In males, testosterone treatment attenuated induced dendritic atrophy, but dendritic lengths were still reduced almost 40% from normal lengths. In sharp contrast, testosterone treatment of females attenuated induced dendritic atrophy almost completely, resulting in dendritic lengths that were not statistically different from those of normal females.

Previous studies have demonstrated that neither axonal [22] nor dendritic [11,23] transport of BHRP are affected by hormone levels. 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 significant 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 true dendritic atrophy.

Compared to the neuroprotective effect of testosterone we observed previously in male rats [23], testosterone treatment in female rats protected them from induced dendritic atrophy to a substantially greater degree. Immunohistochemical labeling for androgen receptors in male and female rats reveals no sex differences in the distribution, subcellular location of staining, or intensity of staining for androgen receptors in motoneurons [24,25]. The specific binding of testosterone in quadriceps muscles of male and female rats is also similar [7]. However, female rats normally have relatively low circulating levels of testosterone (>2 ng/ml, or about 8% of male levels [13,29]). The implants used in the present study were designed to produce normal male levels of circulating levels of testosterone, and as a consequence, they would have produced substantially higher than normal levels of testosterone in the female rats. Interestingly, as reported in other models of motoneuron injury [20], the degree of protection from induced atrophy after partial motoneuron depletion with testosterone treatment is dose-dependent [6]. Thus, it is possible that the enhanced effect of testosterone treatment in females reflects their response to the relatively larger than normal circulating level of testosterone. Alternatively, in our previous study [23], normal males had larger dendritic arbors than the normal females used in this study. Dendritic lengths in testosterone-treated saporin males [23] or females (current study) were both approximately 6000 μ m per motoneuron. Thus, it is possible that this length reflects the degree of attenuation of atrophy in quadriceps motoneurons that can be achieved with this dose of testosterone, resulting in a greater protective effect in females. However, treatment of males with dosages of testosterone twice that used in the current study does not result in a greater attenuation of induced atrophy [6].

Androgens have robust neuroprotective effects on motoneurons, mediating a variety of cellular and molecular mechanisms [8]. Establishing which of these mechanisms, proteins, and pathways are involved in the androgen-mediated protection of motoneuron dendrites from injury-induced atrophy will be valuable contributions to establishing new neurotherapeutic strategies. The present results indicate that androgens are capable of attenuating secondary atrophy in surviving nearby motoneurons in females as well as males, further supporting a role for testosterone as a neurotherapeutic agent in the injured nervous system.

Acknowledgments

We wish to thank Dr. Cara L. Wellman, Tom Verhovshek, and our anonymous reviewers for their helpful comments on the manuscript. This work was supported by NIH-NINDS NS047264 to D.R.S.

References

1. Bisby MA, Tetzlaff W. Changes in cytoskeletal protein synthesis following axon injury and during regeneration. *Mol Neurobiol* 1992;6:107–123. [PubMed: 1476674]
2. Brännström T, Havton L, Kellerth JO. Changes in size and dendritic arborization patterns of adult cat spinal α -motoneurons following permanent axotomy. *J Comp Neurol* 1992;318:439–451. [PubMed: 1578011]
3. 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]
4. Coggeshall RE. A consideration of neural counting methods. *Trends in Neurosci* 1992;15:9–13.
5. Coons KD, Sengelaub DR. Protection from dendritic atrophy with testosterone following partial motoneuron depletion: Timing and duration of treatment, functional correlates in motor activation, Society for Neuroscience Abstracts Viewer/Itinerary Planner. 2008Program No. 556.23
6. Coons KD, Wilson RE, Sengelaub DR. Protection from dendritic atrophy with testosterone following partial motoneuron depletion: dose-dependence in males and efficacy in females, Society for Neuroscience Abstracts Viewer/Itinerary Planner. 2007Program No. 56.11
7. 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]
8. Fargo KN, Foecking EM, Jones KJ, Sengelaub DR. Neuroprotective actions of androgens on motoneurons. *Front Neuroendocrin* 2009;30:130–141.
9. Fargo KN, Foster AM, Sengelaub DR. Neuroprotective effect of testosterone treatment on motoneuron recruitment following the death of nearby motoneurons. *Dev Neurobiol*. 2009in press
10. Fargo KN, Sengelaub DR. Testosterone manipulation protects motoneurons from dendritic atrophy after contralateral motoneuron depletion. *J Comp Neurol* 2004;469:96–106. [PubMed: 14689475]
11. Fargo KN, Sengelaub DR. Exogenous testosterone prevents motoneuron atrophy induced by contralateral motoneuron depletion. *J Neurobiol* 2004b;60:348–359. [PubMed: 15281072]
12. 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]
13. Frankel A, Mock EJ, Wright WW, Kamel F. Characterization and physiological validation of a radioimmunoassay for plasma testosterone in the male rat. *Steroids* 1975;25:73–98. [PubMed: 1111175]
14. 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]
15. 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]
16. 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]
17. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. *Neurology* 1968;18:671–680. [PubMed: 4233749]
18. Kochakian CD. Definition of androgens and protein anabolic steroids. *Pharmac Therap B* 1975;1:149–177.
19. Kochakian CD, Tillotson C, Austin J. Castration and the growth of muscles in the rat. *Endocrinol* 1956;58:226–231.
20. 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]
21. 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]

22. 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]
23. 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]
24. 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]
25. 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]
26. Mesulam, MM. *Tracing Neural Connections with Horseradish Peroxidase*. Wiley; Chichester: 1982. p. 268
27. 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]
28. 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]
29. 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 Env Heal* 1978;4:785–803.
30. Osborne MC, Verhovshek T, Sengelaub DR. Androgen regulates trkB expression in spinal motoneurons. *J Neurosci Res* 2007;85:303–309. [PubMed: 17131419]
31. 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. 2006Program No. 683.12
32. Smith, ER.; Damassa, DA.; Davidson, JM. Hormone administration: peripheral and intracranial implants. In: Meyer, RD., editor. *Methods in Psychobiology*. Academic Press; New York: 1977. p. 259-279.
33. Titmus MJ, Faber DS. Axotomy-induced alterations in the electrophysiological characteristics of neurons. *Prog Neurobiol* 1990;35:1–51. [PubMed: 2217820]
34. Wainman P, Shipounoff GC. The effects of castration and testosterone propionate on the striated perineal musculature in the rat. *Endocrinol* 1941;29:975–978.

Figure 1.

Weights of the vastus medialis (top) and vastus lateralis (bottom) muscles in normal females and saporin-injected females that were either untreated (SAP) or treated with testosterone (SAP +T). Gray bars represent weights from the right (R) and left (L) sides in normal females, filled bars represent weights from the untreated contralateral (right) side of the quadriceps muscle, and unfilled bars represent weights from the saporin injected (left) side of the quadriceps muscle. Bar heights represent means $\pm$ SEM. * indicates significantly different from normal females.

Figure 2.

(A) Darkfield digital micrographs of transverse hemisections through the lumbar spinal cords and corresponding computer-generated composites of BHRP-labeled somata and processes of a normal female (top), a saporin-injected female (SAP, middle), and a testosterone-treated saporin female (SAP+T, bottom) after BHRP injection into the left vastus lateralis muscle. Scale bar = 500 μ m. (B) Dendritic lengths of quadriceps motoneurons from the same groups as above, expressed as arbor per cell. Bar heights represent means $\pm$ SEM. * indicates significantly different from normal females.