Sex Differences in the Motor Nucleus of Cranial Nerve IX-X in *Xenopus laevis*: A Quantitative Golgi Study

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SUMMARY

In the clawed frog (*Xenopus laevis*), motor neurons in cranial nerve nucleus IX-X control contraction of laryngeal muscles responsible for sexually dimorphic vocal behaviors. We examined sex differences in dendritic arbors of n.IX-X cells using the Golgi–Cox method. Three morphological classes of somal types (ovoid, triangular, and elongate) are present in similar frequencies in n.IX-X of both males and females. The male n.IX-X neuron is a more complex and hypertrophied version of the female n.IX-X cell. The number of primary dendrites is the same for both sexes, but males have more total dendritic segments. The overall dendritic length of male n.IX-X neurons is two to three times that of the female. Males have longer dendritic segments between all branch points.

Male and female frogs differ in levels of circulating androgens; neurons of n.IX-X are targets for androgenic steroids. To determine if androgen can affect dendritic morphology in adult females, we examined Golgi-impregnated cells in n.IX-X from ovariectomized females treated with testosterone for 1 month. The total number of dendritic segments was reduced by androgen treatment due to reduction in the number of higher order dendritic segments; the number of primary dendritic segments was unchanged. Androgen treatment may induce resorption of higher order dendritic branches. The overall dendritic length of androgen-treated female n.IX-X neurons was unchanged, and dendritic segments were longer. Thus, although androgen can alter dendrites of n.IX-X cells in adult females, this short-term treatment does not produce a masculine dendritic architecture.

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INTRODUCTION

The successful execution of many reproductive behaviors depends on the action of gonadal steroids on specific target cells in the nervous system. Neither the precise physiological effects of hormones nor their mechanism of action is well understood. Hormones could change synaptic connectivity or efficacy within the neural circuitry of a particular behavior by altering the dendritic architecture of hormone target cells (Greenough et al., 1977; DeVoogd and Nottebohm, 1981a,b). In some species, neurons in specific nuclei of male and female brains differ in size and dendritic morphology (Raisman and Field, 1971; Nottebohm and Arnold, 1976; Gorski et al., 1978; Breedlove and Arnold, 1983a). During early development, the secretion of male-typical sex hormones can induce permanent masculinization of certain brain nuclei (Raisman and Field, 1973; Gurney and Konishi, 1980; Breedlove and Arnold, 1983b). In adulthood, reversible changes in neuronal size and dendritic extent can occur in response to fluctuations in circulating hormone levels (Nottebohm, 1980; DeVoogd and Nottebohm, 1981b; Gurney, 1981; Kurz et al., 1986).

The South African clawed frog, *Xenopus laevis,* exhibits sexually dimorphic vocal behaviors (see Kelley, 1986, for review). Males use mate calls—repetitive, amplitude-modulated trills—to attract females (Wetzel and Kelley, 1983). Females do not mate call but use a different vocal behavior—ticking—to repel or terminate a male clasp attempt (Weintraub et al., 1985). Males require circulating androgen to produce mate calls, while females cannot be induced to mate call even when given exogenous androgen (Wetzel and Kelley, 1983; Hannigan and Kelley, 1986). Thus the sexes may differ in androgen sensitivity or in the neural and muscular effectors for mate calling. Mate calls and ticking are produced by contraction of the laryngeal dilator muscles (Yager, 1982; Tobias and Kelley, 1987), which are innervated by motor neurons in cranial nerve nucleus IX-X (n.IX-X) of the caudal medulla (Kelley, 1980; Simpson et al., 1986). Both muscles and motor neurons contain androgen receptors in both sexes (Kelley, 1981; Segil et al., 1987). Thus females maintain the intracellular protein necessary for androgen responsiveness. However, the neurons of n.IX-X are more numerous and larger in adult males than in females (Hannigan and Kelley, 1981; Simpson et al., 1986), as are the laryngeal muscles (Sassoon and Kelley, 1986). These sex differences are accompanied by differences in androgen secretion. Circulating levels of androgen are greater in adult males than in females (Kelley, 1980). Sex differences in androgen levels first appear during juvenile stages following metamorphosis (Lambdin and Kelley, 1986). Based on work in rodents (Gorski et al., 1978; Breedlove and Arnold, 1983a,b) and song birds (Gurney and Konishi, 1980; DeVoogd and Nottebohm, 1981b), we have hypothesized that sex differences in cell number and size in n.IX-X of *Xenopus laevis* are influenced by differences in levels of circulating androgen during development and/or after sexual maturity (Kelley, 1986).

The aim of the present study was to extend our analyses of cellular sexual dimorphisms in n.IX-X to include differences in dendritic architecture. In addition, we wished to assess contributions of short-term elevations in androgen levels in adult females as a first step toward exploring developmental changes in response to androgen. We chose to use the "reazzione nera" of

Golgi to study cell morphology because this method, although capricious, is seldom matched in its ability to reveal fine details of dendritic morphology at the light microscopic level. Previous studies using this method have revealed sex differences in somal size (Gurney, 1981), in number of primary dendrites (Gurney, 1981), in branching patterns of dendrites (Greenough et al., 1977; DeVoogd and Nottebohm, 1981a), and in dendritic lengths (Gurney, 1981; DeVoogd and Nottebohm, 1981a) in a variety of telencephalic and diencephalic nuclei that participate in the control of reproductive behaviors in other species.

METHODS

Tissue from seven intact male, five intact female, and five ovariectomized androgen-treated female *Xenopus laevis* was used in this study. All animals were obtained reproductively mature from Xenopus I (Ann Arbor, MI). In comparing adult males and females, we wished to minimize sex differences in circulating androgen at the time tissue was obtained because we believed that sex differences revealed with relatively low hormonal levels in adult males might provide clues as to cellular changes occurring earlier in development. In the wild, *X. laevis* breeds seasonally; spawning bouts are triggered by changes in rainfall and temperature (Kalk, 1960). In the laboratory under conditions of constant temperature, some elements of this annual cycle persist. For example, females spontaneously become sexually receptive in early July (Weintraub et al., 1985). All intact male and female frogs were thus sacrificed between December and March. None of the males showed any nuptial pad development at the time of sacrifice. Nuptial pads are an androgen-dependent secondary sex characteristic of males. To maximize possible effects of androgen on adult females, we delivered supraphysiological (Wetzel et al., 1985; Lambdin and Kelley, 1986) doses of androgen. The length of treatment was short because longer treatment (followed by gonadotropin injection) appears to reduce the number of cells in n.IX-X (Hannigan and Kelley, 1983), and we did not wish to complicate the interpretation of data by inducing cell loss. Adult females were ovariectomized and implanted with a 20 mg testosterone proprionate pellet in the dorsal lymph sac for 1 month. Such treatment is sufficient to alter female vocal behaviors (Hannigan and Kelley, 1986). The pellet was removed 1 week prior to sacrifice, a protocol used in our previous studies of Nissl-stained n.IX-X neurons (Hannigan and Kelley, 1983). All females displayed robust nuptial pads in response to testosterone treatment.

Each frog was sacrificed by decapitation, and its brain was immediately dissected out and processed with a variant of the Golgi–Cox method described by Ramon-Moliner (1970). Briefly, fresh nervous tissue was impregnated in a solution of mercuric chloride, potassium chromate, potassium dichromate, and sodium tungstate for approximately 3 weeks. Following impregnation, the tissue was washed, alkalinized in a lithium hydroxide, potassium nitrate solution, washed in a weak acetic acid solution, and then dehydrated and embedded in soft Epon for sectioning (120 μm in the transverse plane). In the present study, tissue was impregnated at pH 6.4 for 23–25 days, since these parameters yielded superior impregnations in pilot work. Pilot work had also indicated that the transverse plane produced fewer truncated dendrites than other planes. The horizontal plane, for example, cut many dendrites within a few micrometers of the soma. Sections were then mounted on clean glass slides and cover-slipped with Permount. As is typical for this Golgi method (Ramon-Moliner, 1970), we observed only Golgi-stained dendrites and not axons in this study. However, it is possible that the proximal portion of the axon was stained and mistaken for a dendrite. Such staining could account for our observation (see below) that some Type I (Ia) cells have an extremely short process emerging from the cell body. If this process is the proximal portion of the axon, it stained in only a very limited subset of cells (Type Ia, not Ib, II, or III). Such processes of Ia cells were not included in analyses.

From each male or female brain, three sections—one each from the anterior, middle, and posterior extent of nucleus IX-X—were selected for examination. The anterior and posterior limits of the nucleus were defined relative to the caudal-most root of cranial nerve IX-X (root 4; Simpson et al., 1986) and the first spinal root, respectively. In these sections, all well-impregnated cells which lay within a criterion area corresponding to the location of n.IX-X (see below) and whose dendrites had not been cut within 50 μm of the soma were drawn with the aid of a camera

lucida at a final magnification of 475×. A "well-impregnated" cell is one in which all apparent dendritic processes are deeply stained (black); no cells with pale (grey or brown) dendrites were drawn. Both the left and the right side of the brain were used; no differences were apparent. No attempt was made to reconstruct dendrites from adjacent sections, since we could not follow these reliably. There were no systematic differences in the frequency with which dendrites were truncated by sectioning in different cell types. For both sexes, third-order dendrites were most frequently truncated. In males, 61% of the cells had one or more truncated dendrites, whereas in females 29% of the cells had truncated dendrites. Since, in addition, male dendrites were consistently longer, measurements may have been biased in the direction of decreasing group differences between males and females. Data on dendritic length in n.IX-X thus represent a lower bound for sex differences. Androgen-treated female brains were analyzed in a similar fashion, except that all sections containing n.IX-X neurons were used to increase sample size.

We determined the location of nucleus IX-X (containing laryngeal motor neurons) by first preparing reference male, female, and testosterone-treated female brains after HRP injections into the laryngeal dilator muscles (see Kelley, 1980). The boundaries of n.IX-X were determined separately for males and females because laryngeal motor neurons are more lateral in males (Simpson et al., 1986). Rectangular criterion areas were then constructed around the HRP-filled neurons in each sex. Criterion areas were adjusted to maximize the number of HRP-filled neurons and minimize the number of unlabeled cells. Unlabeled cells represent (1) laryngeal motor neurons not exposed, at axon or synapse, to HRP, (2) cells in n.IX-X that are not laryngeal motor neurons, and (3) cells at the extreme lateral edge of nucleus reticularis inferior. The criterion area contained 97% of the female HRP-filled cells, 88% of the male cells, and 99% of the androgen-treated female cells. The location of the nucleus is shown in Fig. 1. For each Golgi-stained brain, the size of the rectangular criterion area was adjusted using the ratio of the cross-sectional area of the entire Golgi section to the cross-sectional area of the entire HRP reference section. In a similar fashion, the rectangular criterion area was placed within the Golgi-stained section using linear ratios of dorsal-ventral and medial-lateral position. The cross-sectional areas of entire transverse sections containing n.IX-X in male and female medullas were virtually identical (ratio of average male to female areas from Golgi-stained sections: 0.99).

Three morphological classes of neurons within n.IX-X could be distinguished (see Results). Each well-impregnated neuron was assigned to the appropriate class and drawn using a camera lucida attached to a Zeiss microscope. Each neuron was then scored for number of processes emerging from the soma (number of primary dendritic segments) as well as the total number of dendritic segments. Measures of somal cross-sectional area, combined length of primary processes (from soma to first branch point), and the cumulative length of all dendritic segments (from soma to termination or edge of section) were taken for each cell using a computer-interfaced bit pad (Zeiss MOP-3 or Morph II, L. Peachey, design).

A more detailed quantitative analysis on individual neurons was performed. Each dendritic segment was assigned a branch order one greater than the number of proximal dendritic bifurcations. At least 21 successfully impregnated neurons from each group and class were measured, or, if there were less than 21 impregnated neurons of a particular class and group, then all cells of that group and class were measured. For each cell examined, the length of dendritic segments was measured, and average lengths for each branch order present in that cell were calculated. The means and variability of somal size and of cumulative primary and total process length from this subset of data were similar to the means and variability of the total data set. Data on number and length of all dendritic segments and on somal area were compared, by cell type, for males, females, and androgen-treated females using an analysis of variance (two-level nested ANOVA with unequal sample sizes; Sokal and Rohlf, 1981). Posthoc comparisons between groups were carried out using the Tukey–Kramer procedure (Dunnett, 1980). The significance of differences between testosterone-treated females and intact females in percentages of cells with higher order dendritic branches was evaluated using the Chi-square test.

RESULTS

A total of 413 successfully impregnated cells (males: 136; females: 171; androgen-treated females: 97) were traced using the camera lucida. Counts of the number of well-impregnated neurons in n.IX-X of individual frogs ranged

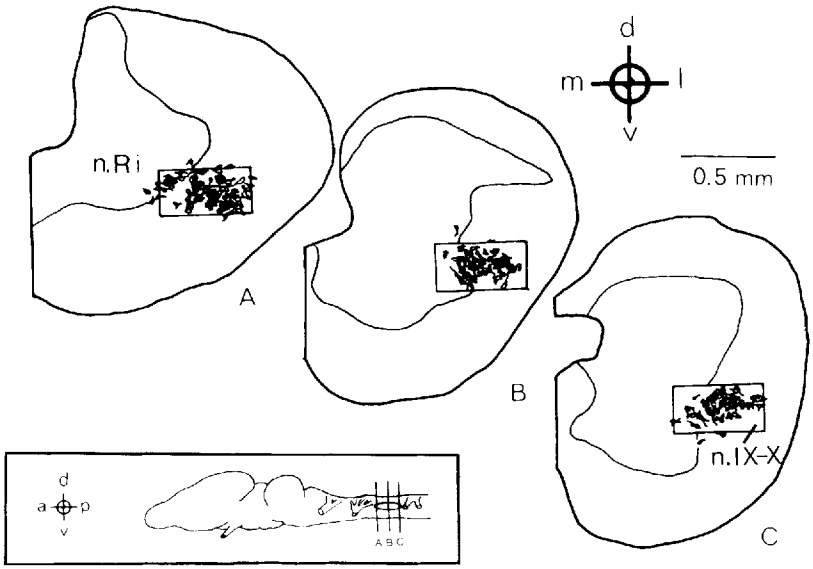

Fig. 1. Locations of n.IX-X neurons as determined by retrograde axonal transport from the m. dilator laryngis. Locations of HRP-filled cells in three transverse sections through the rostral (A), middle (B), and caudal (C) extent of the nucleus are illustrated for a testosterone-treated gonadectomized female sacrificed 2 days after injection of HRP into the larynx. The location of n.IX-X relative to the entire frog brain is illustrated at the lower left. The nucleus extends from a point immediately posterior to the caudal root of the n.IX-X complex (at left) to a point just anterior to the most rostral spinal nerves (at right). Locations of n.IX-X cells were also determined for male and female brains (not shown). The locations of all HRP-labeled cells were projected onto the closest of the three reference sections. A rectangular criterion area encompassing the majority of n.IX-X cells was then constructed and projected, after correction for area and placement, onto Golgi-stained transverse sections through n.IX-X of the appropriate sex and/or hormone treatment. n.Ri: nucleus reticularis inferior.

from 10 to 30 in males (mean: 20), 16 to 55 in females (mean: 37), and 7 to 37 in testosterone-treated females (mean: 23). A small number of cells (5) in n.IX-X of androgen-treated females appeared morphologically abnormal in that cell bodies were very small and processes were few in number. These stunted cells were excluded from analyses.

Morphological Classification of n.IX-X *Cells*

Two groups of efferent neurons send axons to the periphery through the most caudal root of the nerve IX-X complex (Simpson et al., 1986). One group, located laterally in the caudal medulla in nucleus IX-X, innervates the dilator (or bipinnate) muscles of the larynx. The other group, located more medially among cells of the nucleus reticularis inferior, consists of unipolar cells that are presumed to be autonomic efferents (Simpson et al., 1986). The majority of Golgi-impregnated cells in n.IX-X were multipolar (Fig. 2). Unipolar neurons were rare (<2%) and were not sexually dimorphic (data not shown). Camera lucida drawings of multipolar cells from both sexes are shown in Figs. 3–6. We

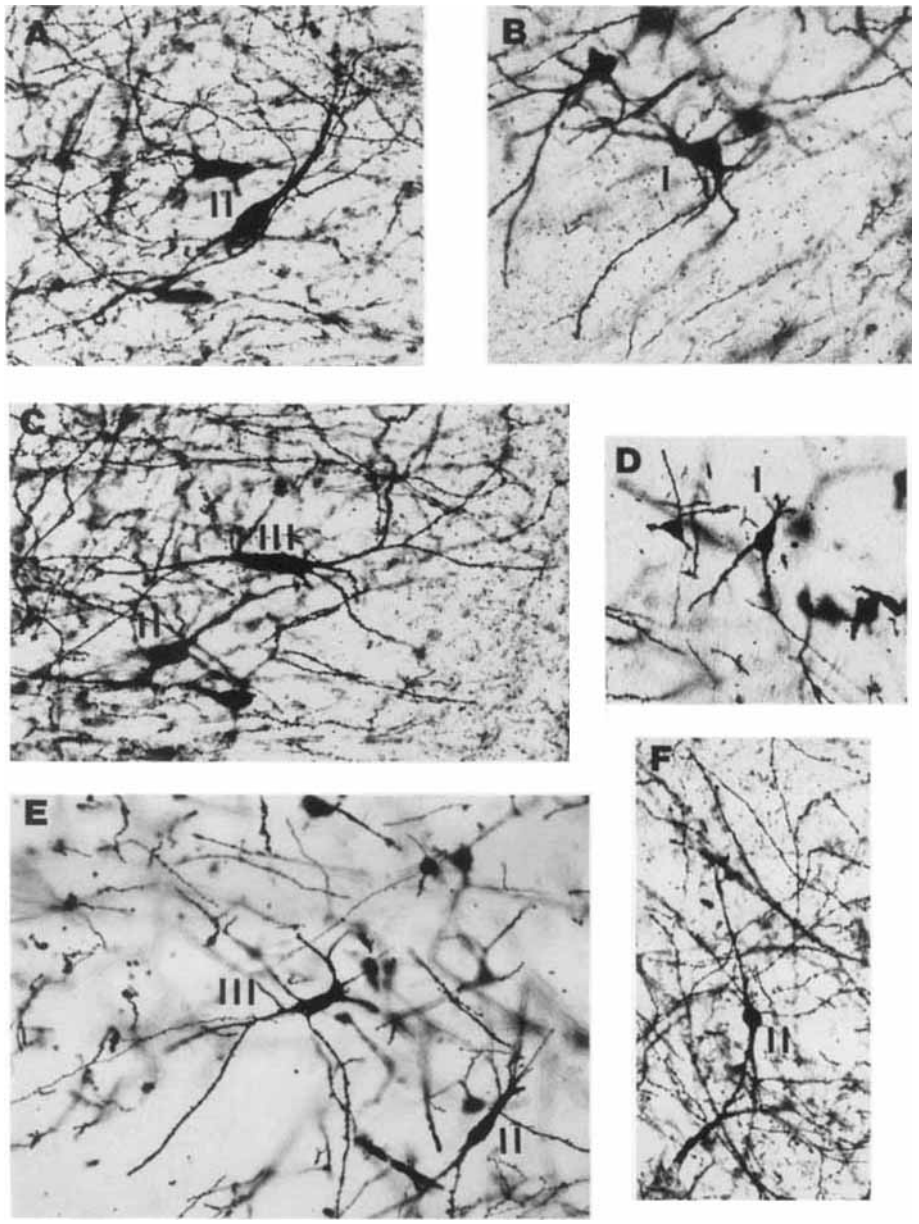

Fig. 2. Photomicrographs of representative cell types in n.IX-X. Golgi-impregnated neurons from males are shown in panels A, B, C, and F and from females in panels D and E. Type I cells are characterized by triangular, Type II by ovoid, and Type III by elongate somata. Note that the entire dendritic tree of the cells is not in focus in photomicrographs of these thick Epon-embedded sections but can be projected onto a planar surface in camera lucida drawings (Figs. 3–6).

classified multipolar n.IX-X neurons into three categories based on somal shape. Type I neurons (average somal area: 362 μm^2) have triangular somata with at least one process emanating from each vertex. The primary dendrites of Type I neurons usually branch symmetrically around the soma. Although

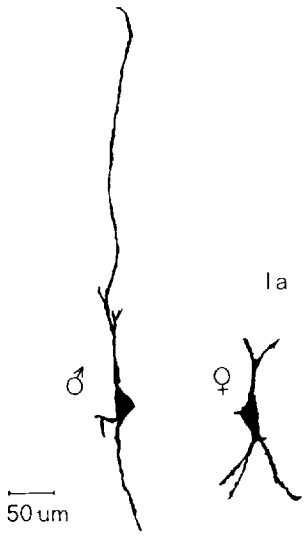

Fig. 3. Camera lucida tracings of representative Golgi-stained Type Ia cells in a male (left) and female (right).

some Type I neurons (Ia; Fig. 3) have one extremely short process (less than 10 μm in length), most are more symmetrical (Ib; Fig. 4). Type II (Fig. 5) neurons have rounded or ovoid somata (mean somal area: 288 μm^2) and an average of three primary dendritic segments. Often two processes emerge about 180° apart, giving the cell a fusiform appearance. There were rare cases (<5%) where cells could be considered borderline between Types I and II; these cells were consistently assigned to the Type II class. Type III neurons (Fig. 6) have elongated and irregularly shaped somata (mean somal area: 560 μm^2) and an average of three primary processes emerging at random orientations. The somata of these neurons often appeared crescent shaped. Although more dorsal and medial cells outside the criterion zone were covered with spines, no spinous dendrites were observed within the HRP-defined motor nucleus of n.IX-X. All cell types were also observed in n.IX-X following HRP injection into laryngeal muscle (see also Simpson et al., 1986).

n.IX-X *Cells in Males and Females*

Male n.IX-X neurons have more total dendritic segments than females (Table 1). This sex difference is attributable to a greater number of higher order dendrites in males because the number of primary dendritic segments is virtually identical to that of females. The mean overall length of the dendritic tree of male n.IX-X cells is 2.3 times that of female neurons. The male/female difference in dendritic segment length is apparent at all orders of dendritic branching (Fig. 7). Only male Type II and III cells exhibit fifth-order branches.

We classified Golgi-impregnated cells by somal shape (I, triangular; II, ovoid; III, crescent shaped). The frequency of different cell types was similar

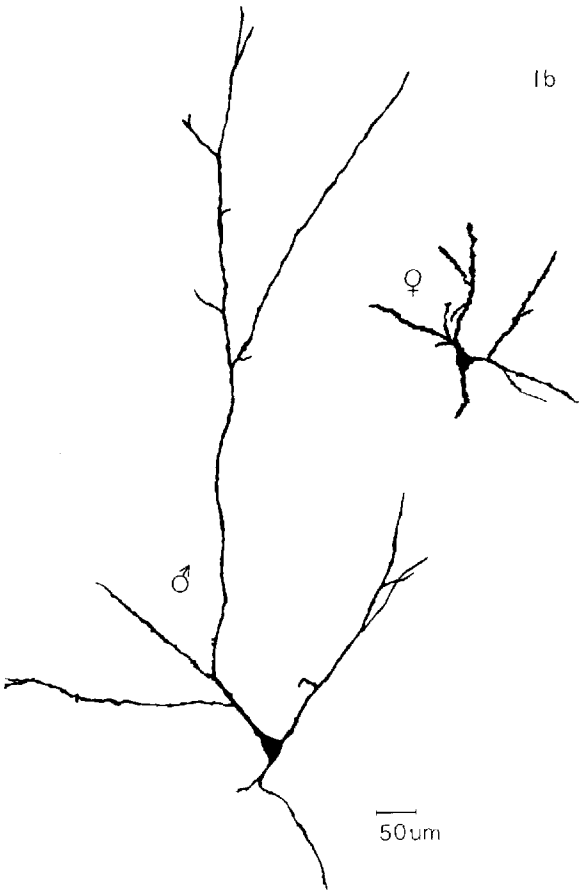

Fig. 4. Camera lucida tracings of representative Golgi-stained Type Ib cells in a male (left) and a female (right).

for the sexes. Cell type contributed significantly to measures of somal area and total dendritic length (Table 1), indicating that cells differing in somal shape also differ in other morphological aspects. For example, Type III cells had the largest somata (Table 1, Type I or II versus Type III cells for both sexes; $p < 0.05$, Tukey–Kramer posthoc test). The total dendritic length of male Type III cells was longer than that of Type I cells ($p < 0.01$, Tukey–Kramer posthoc test). Only Type II and III cells had fifth-order branches (and then only in males; Fig. 7). There were no significant differences in somal area between the sexes.

Effects of Androgen Treatment

All morphological classes of multipolar n.IX-X cells were recognizable in testosterone-treated females (see Fig. 8 for examples). Frequencies of Type I, II, and III cells in androgen-treated females were similar to those seen in intact females as were the mean number of primary dendritic segments (Table 1). Somal areas did not differ significantly from untreated females. Testoster-

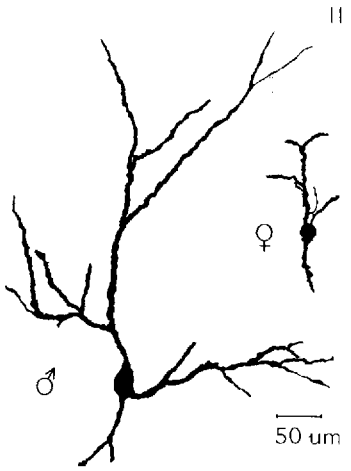

Fig. 5. Camera lucida tracings of representative Golgi-stained Type II cells in a male (left) and a female (right).

one treatment, however, significantly decreased the total number of dendritic segments while leaving total dendritic length unaffected. The number of Type I and Type III cells with higher order branching in testosterone-treated females was significantly less than in untreated females (Fig. 9). Androgen treatment did not significantly affect this measure in Type II cells. On average, higher order dendritic branches were 23% longer in testosterone-treated females.

DISCUSSION

Neurons in n.IX-X of males have longer and more numerous dendritic segments than female cells. The sex difference in dendritic length is apparent at all orders of dendritic branching. The number of primary dendritic seg-

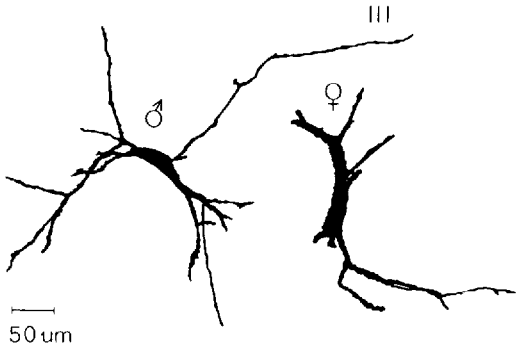

Fig. 6. Camera lucida tracings of representative Golgi-stained Type III cells in a male (left) and a female (right).

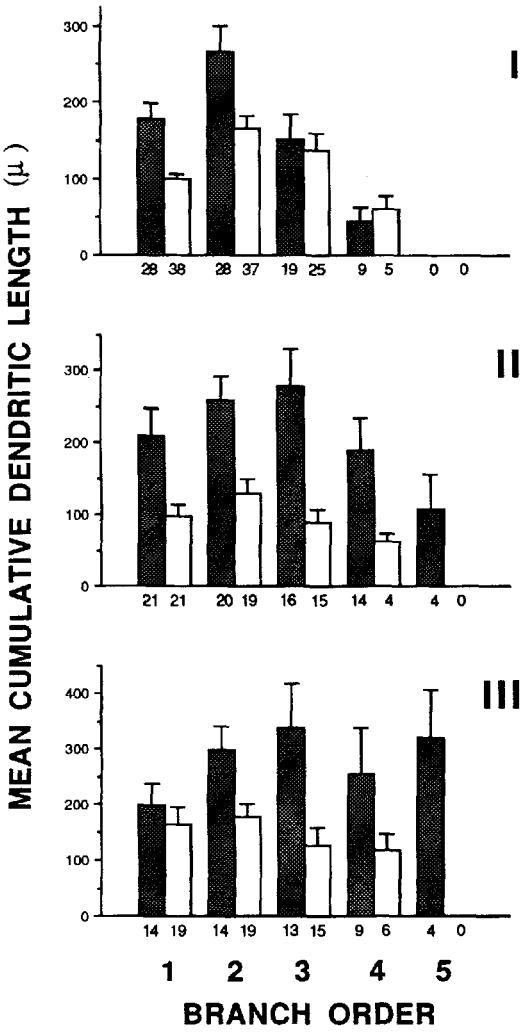

Fig. 7. Sex differences in mean cumulative dendritic length for different orders of dendritic branching by neuronal type. For each cell of a given type in males and females, the cumulative length of primary, secondary, tertiary, quaternary, and quintenary dendrites was measured and then averaged for each branch order. Values shown are means ± SEM. Shaded bars: male; open bars: female. Sample size for each branch order is indicated beneath each bar.

ments, however, is the same in males and females. The greater number of dendritic segments in males is thus attributable to a greater number of higher order branches. Since there is no sex difference in the frequency of n.IX-X cell type, we suggest that the male n.IX-X cell is a more complex and hypertrophied version of the equivalent female cell. The sex difference in the n.IX-X neuron dendrite length in *X. laevis* is similar in magnitude to the two-fold difference reported in zebra finches (Gurney, 1981) for one type of cell in

TABLE 1

Percentage of Total Cells within Each Sex, Mean Somal Area (μm^2), Mean Number of Primary Dendritic Segments/Cell, Mean Total Number of Dendritic Segments/Cell, Mean Cumulative Length of Primary Segments/Cell, Mean Total Length of Dendritic Segments/Cell for Type I, II, and III and Male (M), Female (F), and Androgen-treated Female (TF) Neurons

Somal	Percent Sex Total			Somal Area			Number of Primary Segments			Total Number of Dendritic Segments			Cumulative Length of Primary Segments			Total Length of Dendritic Segments		
Type	M	F	TF	M	F	TF	M	F	TF	M	F	TF	M	F	TF	M	F	TF
I	28	33	35	382	342	468	3.2	3.0	3.2	12.5[a]	8.5[b]	5.6	178	99[b]	187	644[c]	362	333
	(38)	(56)	(32)	± 23	± 20	± 34	± 0.1	± 0.1	± 0.1	± 1.1	± 0.6	± 0.6	± 20	± 6	± 21	± 50	± 50	± 39
II	57	50	41	321	275	379	3.2	3.1	2.7	12.8[a]	8.5	6.9	186[c]	114	120	814[a]	292	315
	(78)	(86)	(38)	± 21	± 9	± 22	± 0.1	± 0.1	± 0.2	± 0.8	± 0.6	± 0.5	± 18	± 7	± 13	± 98	± 42	± 31
III	15	17	24	580	540	706	3.6	3.7	3.0	13.9	11.5[d]	6.4	214	139	221	1067[a]	452	395
	(20)	(29)	(22)	± 104	± 79	± 59	± 0.2	± 0.2	± 0.2	± 1.2	± 0.6	± 0.5	± 35	± 24	± 41	± 145	± 52	± 53

Note: Significance of differences was evaluated using a two-level nested ANOVA. There was a significant contribution of cell type to somal area ($p < 0.001$), number of segments ($p < 0.01$), and total dendritic length ($p < 0.001$). There were no significant differences between groups for somal area and number of primary segments. Total number of dendritic segments ($p < 0.005$) and dendritic length ($p < 0.01$) differed significantly between groups. Values are means ± standard error; number of cells is given in parentheses. Significance of differences was evaluated with a Tukey–Kramer posthoc test.

[a] M versus F, $p < 0.01$.

[b] F versus TF, $p < 0.05$.

[c] M versus F, $p < 0.05$.

[d] F versus TF, $p < 0.01$.

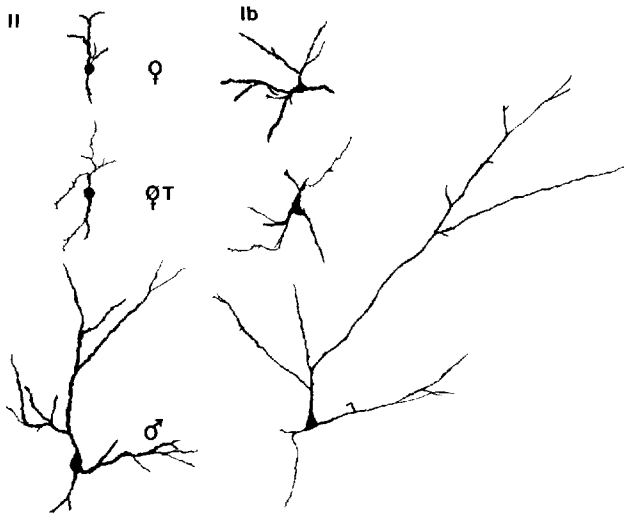

Fig. 8. Effects of testosterone treatment on the morphology of n.IX-X cells in gonadectomized females illustrated for two cell types (II and Ib). For comparison, camera lucida tracings of equivalent cell types in intact males (lower panels) or intact females (upper panels) are shown. Bar = 50 μm.

telencephalic nucleus RA. The greater overall length of RA dendrites in male zebra finches, however, is due to three factors: an average of seven primary dendrites as opposed to four in the female, more dendritic segments, and longer dendritic segments.

Based on somal shape, we can distinguish three classes of cell in n.IX-X. Our analyses indicate that cell type contributes significantly to differences between groups. We do not know at present whether these morphological categories of n.IX-X cells correspond to functional cell types. Since neurons that provide innervation to laryngeal muscle come in all three shapes, we cannot distinguish laryngeal motor neurons from other cells simply by shape. Approximately half of the cells in n.IX-X accumulate androgen (Kelley, 1980). It is possible that one cell type in the nucleus is the androgen-concentrating cell as has been reported for dimorphic neurons in the zebra finch song system (Nordeen et al., 1987). Other possibilities for functional differences include different afferent inputs and synaptic targets. Sex differences in dendritic segment number and length are not due to differences in the frequency of a particular cell type (I, II, or III).

In a previous study of n.IX-X (Hannigan and Kelley, 1981), we observed a sex difference in somal size of Nissl-stained neurons. In Golgi-impregnated material, however, there is no significant difference between somal sizes in the sexes. This difference in results may be due to differences in adult hormonal status; males had, presumably, low levels of androgen in this study, whereas androgen levels were not controlled in our previous comparison. Differences in results could also be due to methods for measuring cross-sectional area. For cresyl-violet-stained cells, the entire stained portion of the cell is measured. Since rough endoplasmic reticulum (stained due to its RNA content) can

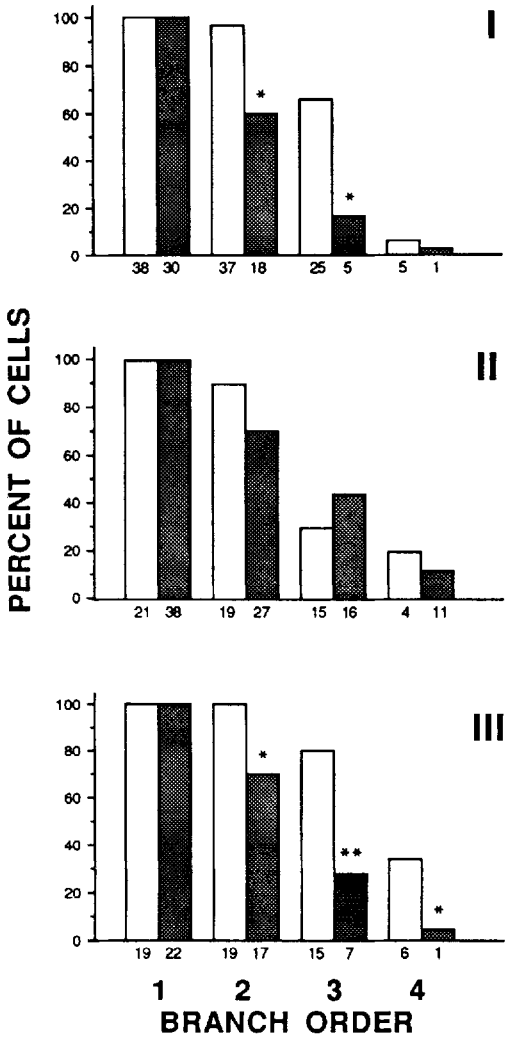

Fig. 9. Effect of testosterone treatment on the frequency of higher order dendritic segments in adult, gonadectomized females. The percentage of cells with higher order branches is reduced by testosterone in all except Type II cells. $*p < 0.05$; $**p < 0.01$, Chi-square test. Open bars: female; shaded bars: testosterone-treated female. Number of cells is indicated beneath each bar.

extend into the proximal portion of primary dendrites, we may have overestimated the extent of the cell body in Nissl-stained material, particularly in males, if androgen increases protein synthesis. The sex difference in somal size of cresyl-violet-stained n.IX-X neurons would thus be due to inclusion of the proximal portion of primary dendrites which are, as we show here, sexually dimorphic in length.

Differences in dendritic extent may contribute to the sexually dimorphic

functions of laryngeal motor neurons by providing a basis for sex differences in connectivity or in the magnitude of synaptic input. We have shown that the temporal patterns that drive mate calling or ticking, the dimorphic vocalizations of *X. laevis,* originate in the central nervous system and are conveyed to laryngeal muscles by n.IX-X neurons (Tobias and Kelley, 1987). Major afferent input to n.IX-X arises from the adjacent inferior reticular formation and a more anterior nucleus, the pretrigeminal nucleus of the dorsal tegmental area (DTAM) (Wetzel et al., 1985). The greater dendritic length and number of dendritic segments in males suggests that male n.IX-X cells may have available a greater postsynaptic dendritic target for these afferents. Dendrites of n.IX-X cells ramify anterior-posteriorly, within the nucleus, and medially, toward adjacent reticular neurons (Simpson et al., 1986). It is possible that the dendritic arbor of male n.IX-X cells may extend into regions, particularly the adjacent reticular formation, not invaded by female cells. Such an extension could attract synaptic input different in quantity or quality from that received by female neurons. Electrophysiological studies indicate that laryngeal motor neurons have sexually dimorphic properties which contribute to sex differences in vocalization (Tobias and Kelley, 1988). Sex differences in dendritic arbors could contribute to the generation of dimorphic vocal patterns and to sex differences in electrophysiological function of vocal motor neurons.

We do not know when sex differences in the dendritic arbors of n.IX-X cells first appear during development. Steroid hormones have been shown to influence the development of dendritic arbors in neurons that contain specific intracellular hormone receptors (target cells; Toran-Allerand, 1976; Gurney, 1981). It is possible that the sex differences in dendritic arbors of n.IX-X cells reflect differences in the endocrine milieu in which male and female cells develop. Autoradiographic studies have shown that androgen receptors are first detectable in cells of n.IX-X at tadpole stage 64 (Gorlick and Kelley, 1986). Males and females exhibit differences in androgen secretion during development. Males begin to secrete more androgen than females starting at 3 months after metamorphosis, reaching adult values at 6 months (Lambdin and Kelley, 1986). Under the influence of androgen, developing male cells may elaborate more and longer dendritic segments than female cells. Since primary dendrites of n.IX-X cells are not sexually dimorphic in number, either this order of dendritic branch is not sensitive to androgen or, more likely, has emerged from the cell body before the onset of sex differences in androgen levels or the expression of androgen receptor.

Two kinds of cell–cell interaction have been implicated in dendritic growth. For some neurons, growth of the dendritic tree is associated with growth of synaptic targets (Purves and Hadley, 1985). For others, the amount of afferent input appears to control dendritic length (Globus and Scheibel, 1967). Androgen targets in *X. laevis* include nucleus DTAM and inferior reticular formation (Kelley et al., 1975; Kelley, 1981) in the CNS and laryngeal muscle (Segil et al., 1987). Sex differences in dendritic arbors of n.IX-X cells could be due to the direct effects of androgen on n.IX-X and/or indirect effects mediated by afferents or synaptic targets.

Previous studies had indicated that androgen has only a very limited ability to masculinize the vocal system of adult female *X. laevis* (Hannigan and Kelley, 1986; Tobias and Kelley, 1987, 1988). We thus sought to determine

whether the number and length of dendrites in n.IX-X of adult females could be increased by exposure to androgen. Results of 1 month of treatment indicated no overall increase in total dendritic length compared with intact females. The number of higher order dendritic segments, however, was decreased, and remaining segments were longer. The decrease in segment number suggests that this testosterone treatment regimen induced loss, perhaps by resorption, of higher order dendritic segments. The increase in the length of remaining segments might be due to such a loss. For example, if a dendritic segment bearing one short branch lost that branch, the apparent length of such a segment would increase without much effect on total length. Alternatively, the androgen treatment administered may have induced some lengthening of remaining dendritic branches. We conclude that this short-term androgen treatment does not masculinize the dendritic arbor of adult female n.IX-X neurons.

In our studies of Nissl-stained n.IX-X neurons, we observed that long-term (4-month) androgen treatment accompanied by gonadotropin injection resulted in a decreased number of n.IX-X neurons in adult, ovariectomized females (Hannigan and Kelley, 1983). However, 4 months of androgen treatment without gonadotropin did not result in fewer n.IX-X cells (Hannigan and Kelley, unpublished). We thus do not believe that data interpretation in this Golgi study is complicated by actual cell loss. On the other hand, the androgen treatment we used may have been harmful to n.IX-X cells. For example, some cells appeared "stunted," with few processes and small cell bodies. Resorption of dendrites inferred from the data may have been due to such damage. Any process-lengthening effect of androgen could be masked by such effects. Additional studies—in which amount and duration of androgen treatment are systematically varied—are required before these issues can be resolved. In the absence of such studies, we cannot rule out the possibility of a more dramatic augmentation of the dendritic arbor. Some motor neurons in other systems are known to be more sensitive to androgen than are n.IX-X cells. For example, dendritic arbors of RA neurons in canaries will grow in response to short-term androgen treatment, even in adulthood (DeVoogd and Nottebohm, 1981a). In rats (Kurz et al., 1986), male SNB neurons contract or maintain their dendritic arbors in response to a month of castration without or with androgen replacement, respectively. We do not yet have evidence for this degree of plasticity in adult n.IX-X cells of *X. laevis*. Laryngeal motor neurons may be responsive to androgen only when immature.

In summary, results of this study show that male and female neurons in the vocal motor nucleus IX-X differ markedly in dendritic extent. Sex differences in the dendritic extent of n.IX-X neurons reflect the sexual differentiation of the neuroeffector unit for vocalization. How do differences in elements of the vocal circuitry contribute to sexually dimorphic vocalizations? How does androgen control the differentiation of target neurons and muscles to effect masculinization? Male and female vocal behaviors in *Xenopus laevis* are produced by a well-characterized efferent pathway and are under strict control of steroid hormones both during development and in adulthood. All developmental stages are readily accessible to experimental manipulation. These features provide powerful advantages for addressing both questions.

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