membrane glycoprotein. Reduction of the disulfide bridges in gastric mucus markedly alters its physical state by converting it from a water-insoluble gel to a water-soluble form (*8*). Derivatives of the sulfhydryl-containing amino acid cysteine have been shown to liquefy mucus (*13*). However, if mucus plays a role in gastric cytoprotection, its optimal form, gel or liquid, is not known. Another possibility is that tissue thiols mediate cytoprotection by enhancing prostaglandin synthesis or inhibiting prostaglandin breakdown.

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10. Since there is diurnal variation in the concentration of nonprotein sulfhydryls in tissue, all animals were killed between 9 and 11 a.m. Mucosa in the glandular stomach was washed with saline and then removed by scraping with a blunt knife. Protein and nonprotein sulfhydryls were measured as described by J. Sedlack and R. Lindsay [*Anal. Biochem.* **24**, 192 (1968)]. Gastric mucosa and liver samples were placed in 40 volumes of 0.2*M* aqueous EDTA and homogenized at 50 rev/min for 6 seconds. Ellman's reagent was added and the concentration of sulfhydryls was determined by measuring the absorbance at 412 nm. All assays were performed in duplicate. Results were calculated as concentration of protein and nonprotein sulfhydryls per gram of tissue.
11. The gastric mucosal concentration of protein and nonprotein sulfhydryls and disulfides was measured by a highly sensitive method modified from A. F. S. A. Habeeb [*Anal. Biochem.* **56**, 60 (1973)] and J. Isaacs and F. Binkely [*Biochim. Biophys. Acta* **497**, 192 (1977)]. Because of the sensitivity of this method, the nonprotein sulfhydryl levels are slightly higher than those obtained by the procedure of Sedlack and Lindsay (*10*).
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Gonadal Hormones Induce Dendritic Growth in the Adult Avian Brain

Abstract. *Ovariectomized adult female canaries were treated with physiological doses of testosterone, dihydrotestosterone, or estradiol. Singing, which is typical of males, occurred in the testosterone-treated birds but not in any of the other birds. The effect of these hormones was assessed on dendrites from a class of neurons in the nucleus robustus archistriatalis (RA), a forebrain nucleus for song control. The RA neurons of the testosterone-treated birds had dendritic trees resembling those of intact males. The RA neurons of the estradiol- and dihydrotestosterone-treated birds resembled those of intact females. All hormone-treated groups had dendrites that were significantly longer than those of untreated ovariectomized females. Thus gonadal hormones induce dendritic growth in the adult avian brain.*

Plasticity in the central nervous system is often thought to be a property of the very young brain. In the development of sexually dimorphic neural systems, the role of steroid hormones is believed to be most powerful at early stages (*1*). We here report experiments that show that steroid hormones can also play a powerful role in inducing neural plasticity in the adult brain.

The canary song system offers the possibility of relating differences in the adult hormonal milieu to altered neuronal organization and changed behavioral capabilities. Several discrete brain nuclei are responsible for song control in this species (*2*). Normally, males sing and females do not. This behavioral dimorphism is reflected in the volume of brain song control nuclei (*3*). Sexual dimorphism is also present at the microanatomical level. We have recently shown that the length and distribution of dendrites of cells in a telencephalic song control nucleus, the nucleus robustus archistriatalis (RA), are highly dimorphic in adult male and female canaries (*4*, *5*). The overall volume of RA increases after testosterone administration to adult females (*6*), a treatment that also induces females to sing in a male-like manner (*6*).

The present experiment was planned to assess neuronal consequences of administering gonadal hormones to ovariectomized adult female canaries, and to relate these effects to the microanatomical configuration seen in intact adult male and female canaries. We studied in detail a neuron type with long, sinuous, spiny dendrites within RA. These cells, which stain reliably and can easily be distinguished from other cell classes within RA, were the object of our previous work assessing neuroanatomical sex differences in intact canaries (*5*).

Thirty-six female canaries were ovariectomized between 12 and 16 days after hatching. When they were 11 months of age we implanted in each bird a Silastic tube containing testosterone, dihydrotestosterone (DHT), estradiol (E), or nothing (ovariectomized control) (*7*). Three weeks later we made tape recordings of the birds that were singing. At 4 weeks after implantation the birds were killed and their brains were removed and stained by a rapid Golgi procedure (*5*). The brains were sliced at 100 μm in the plane of Stokes *et al.* (*8*). Cells of the type in which we were interested were found in RA in the brains of six testosterone-treated birds and seven each of the E- and DHT-treated birds and the ovariectomized controls. A computer-microscope system was used to record the coordinates of at least ten cells each from the right and left hemispheres of the brains in each group. As in earlier studies comparing cells of this class from male and female canaries (*5*), we described all dendritic branches, including those in adjacent tissue sections, using three-dimensional coordinates. We then analyzed the data by measuring dendritic branch lengths at different orders of branching from the cell body and by the Sholl concentric sphere analysis (*9*).

Each of the testosterone-treated ovariectomized females sang vigorously. No other ovariectomized female sang. However, in studies of hormone dosages we observed that six intact females treated with DHT-filled Silastic tubes like those used in the present study also sang vigorously. Thus testosterone, or a combination of its metabolites E and DHT, seems

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necessary for song induction in adult females.

Dendritic branch length and numbers were substantially different in neurons from birds treated with different hormones. Dendrites of cells from the ovariectomized females without hormone implants ended closest to the cell body and had the smallest number of branches of all the groups measured (Fig. 1 and Table 1). The Sholl analysis indicted that the peak number of intersections with a Sholl sphere also occurred closer to the soma in this than in any of the other groups (Fig. 2). In contrast, dendrites from testosterone-treated birds had the largest total dendritic length and the greatest number of branches (Table 1); in these cells, the peak number of intersections with a Sholl sphere was nearly as far from the soma as in cells from intact male canaries. Dendrites from the E- and DHT-treated birds had dendrites with properties that closely resembled each other and were intermediate to those shown by the ovariectomized controls and the testosterone-treated birds. These relations were evaluated statistically by comparing the median Sholl intersection value (the distance from the cell body at which half the intersections are nearer to the cell body and half are further from it) for each group (*10*). As shown in Table 1, cells from both E- and DHT-treated groups have significantly greater median values than do cells of untreated birds. Values for the cells from E- and DHT-treated birds are not significantly different from those of cells from intact females. The values for cells from testosterone-treated females are significantly greater than those of cells from the E- and DHT-treated females and the intact females, and are similar to the values obtained from intact males.

Detailed examination of dendritic branch numbers and lengths (Table 1) revealed almost no differences between

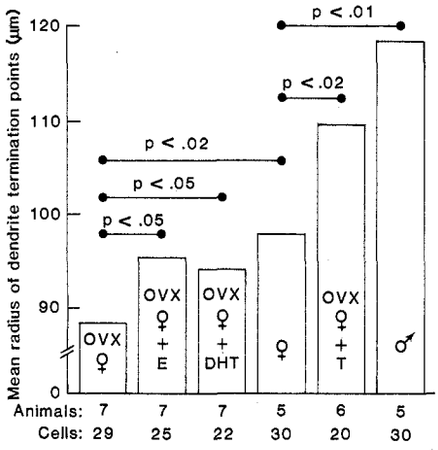

Fig. 1. Dendritic field size related to hormonal treatment. Dendritic field size for each cell was estimated by averaging the distances from all dendritic termination points to the cell body. *OVX*, neonatally ovariectomized females.

groups at the level of first and second order branching. Substantial differences in number and length of branch segments only occur more peripherally. Thus, cells from testosterone-treated females have 95 percent as many first- and second-order branches as do cells from untreated ovariectomized females, but have 150 percent as many branches more peripherally. One consequence of these differences is an expansion of the volume of tissue through which a neuron's dendrites course. As shown in Fig. 1, the mean distance from the tip of a dendrite to its cell body showed a progression from the ovariectomized control females to the ovariectomized females treated with E, DHT, and the intact females, and finally to the testosterone-treated ovariectomized females and the intact males. Since the dendritic field of these cells is spheroidal (*11*), this change represents a 2.5-fold increase in the volume encompassed by cells from the testosterone-treated group, potentially allowing each of these larger RA cells to receive input from a wider range of afferents.

Our data suggest that both E and DHT can cause neuronal growth in an adult female canary. The mechanism of this growth is unclear. Although cells in RA are known to accumulate androgen, no evidence has been found for RA estrogen receptors (*12*). Our data show testosterone to be more effective in inducing

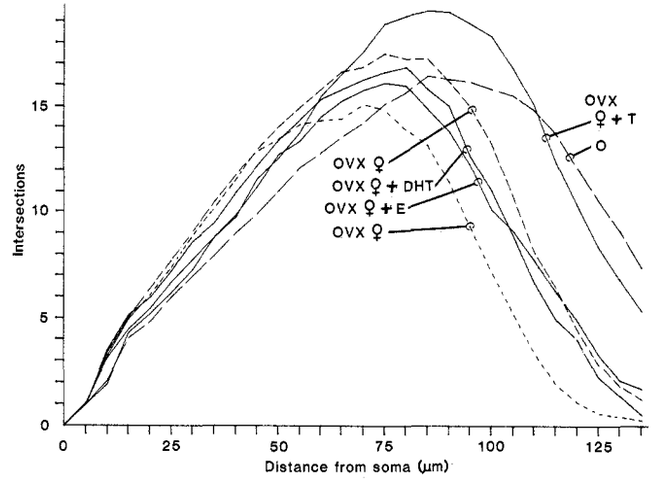

Fig. 2. Sholl analysis of dendritic distribution. For this Sholl analysis, the number of intersections was determined between the dendrites of the RA cells studied and concentric spheres centered on the cell body and separated by 5-μm increments. *OVX*, neonatally ovariectomized females.

Table 1. Effects of hormones administered to adult birds on the dimensions of dendrites. All length measures are in micrometers. Ovariectomies were performed 12 to 16 days after the birds were hatched.

Treatment conditions	Mean number (N) and length (L) of branch segments at each order of branching										Total length (μm)	Sholl median (μm)
	First		Second		Third		Fourth		Fifth			
	N	L	N	L	N	L	N	L	N	L		
Females												
Ovariectomized	4.4	22.7	8.3	44.4	11.0	48.7	7.4	48.3	3.3	39.4	1487	59.9
Ovariectomized plus E	4.1	25.9	7.7	42.8	10.9	50.9	8.3	49.6	4.8	42.1	1641	67.6*
Ovariectomized plus DHT	4.2	27.0	8.0	41.7	11.5	51.5	9.5	45.2	4.8	40.3	1667	65.5*
Intact	4.6	25.1	8.9	47.3	12.1	53.4*	8.7	51.2	5.2	39.6	1817*	67.5*
Ovariectomized plus testosterone	4.1	21.5	8.0	45.7	13.1*	58.1*	12.5*†	56.0*	6.9*	41.9	2213*†	79.5*†
Males (intact)	4.3	29.2	8.3	54.4	12.0	61.4*†	9.8*	54.4	5.4*	40.2	2071*†	83.1*†

*Significantly different from ovariectomized females. †Significantly different from intact females.

dendritic growth than either DHT or E alone (*13*), but we do not know whether this is a result of additive or interactive effects of the metabolites or of specific effects of testosterone itself.

In both birds and rodents, hormonal differences in very young animals result in behavioral and neuroanatomical divergence of the sexes as adults (*14, 15*). In rodents, the incidence of behaviors typical of the opposite sex can frequently be increased by hormone therapy in adulthood (*16*). In the adult female canary, testosterone induces singing, a male-typical behavior, although female song is not as complex as that of males (*6, 17*). We have shown that dimorphic features of one cell class within the song system become male-like in testosterone-treated ovariectomized females. However, although the volumes of song control brain nuclei such as RA grow with androgen treatment, they do not attain male sizes (*6*). Possibly, a smaller number of neurons in telencephalic vocal control nuclei of females (*15*) sets a limit to the size of these nuclei and to the vocal virtuosity they control.

The hormone-induced changes reported here are remarkable in their magnitude and relation to a behavioral change. To our knowledge, this is the first report of dendritic growth induced by gonadal hormones in the adult brain.

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10. Each hemisphere that had well-stained cells was represented by the mean values of all cells sampled from that hemisphere. Preliminary evaluation of these data revealed that no left-right differences were present. Data from cells located in the two hemispheres were therefore pooled for the statistical comparisons. For each comparison described in the present study we used *t*-tests based on animal means. Thus, for each measure, one value was obtained for each animal.
11. When viewed from several perspectives, nearly all of the cells from the intact male and female canaries proved to have spherical or spheroidal dendritic fields. This was so even when the cell soma, because of its proximity to the RA boundary, had an eccentric position within this field. The volume of this field was estimated for each cell by averaging the distances from dendritic tips to the cell body and treating this value as the radius of a sphere.
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The Fish Connection: A Trophic Link Between Planktonic and Rocky Reef Communities?

Abstract. *The blacksmith* (Chromis punctipinnis), *an abundant pomacentrid fish off southern California, regularly forages on zooplankton during the day and shelters in rocky reefs at night. This behavioral pattern results in the importation of 8 grams of carbon per square meter per year, deposited as feces in the nocturnal shelter. Since blacksmiths regularly return to the same shelters, this represents a transport of extrinsic organic carbon to the reef which is predictable in time and space.*

Inshore reef communities obtain organic carbon from two major sources: algae attached to the reef and plankton and detritus transported to the reef by water currents. Oceanic zooplankton may also be a source of energy; Hamner and Carleton (*1*) concluded that their contribution to the reef has not been accurately assessed. We believe that the inaccuracy is due in part to the fact that an important trophic link has been overlooked. In this report we describe a pathway through which oceanic zooplankton, consumed by planktivorous fish, are made available to the benthic community of inshore rocky reefs in a manner that is predictable in time and space.

Many temperate and tropical fish regularly aggregate in the midwater and consume zooplankton (*2–4*). While involvement of planktivorous fish in the importation of energy to reefs has been suggested (*5*), the mechanism has not been investigated. The blacksmith (*Chromis punctipinnis*) is an abundant fish of inshore rocky reefs in southern California (*3, 6*). At dawn blacksmiths emerge from their shelters and migrate to specific locations in the midwater, where they forage almost exclusively on zooplankton; at dusk they return to the reef and shelter in crevices until dawn (*3, 6*). This diel behavior is reflected in a pattern of gut fullness: guts are generally full at dusk and empty at dawn (*3, 6*). The pattern, often used as evidence that planktivores feed during the day (*4*), also indicates that the fish defecate at night while in their shelters.

To determine whether blacksmiths egest feces at night, we collected fish at dusk with the aid of the anesthetic quinaldine and placed them individually into 20-liter plastic buckets that had lids fitted with plankton netting (70-μm mesh) to promote water exchange yet exclude sediments. The buckets were placed at a depth of 10 m along the rocky bottom off Santa Catalina Island, 35 km southwest of Los Angeles, and retrieved the following dawn while most fish were emerging

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