Act locally and think globally: Intracerebral testosterone implants induce seasonal-like growth of adult avian song control circuits

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Edited by Peter Marler, University of California, Davis, CA, and approved July 22, 2002 (received for review May 22, 2002)

There is pronounced seasonal plasticity in the morphology of the neural circuits that regulate song behavior in adult songbirds, primarily in response to changes in plasma testosterone (T) levels. Most song nuclei have androgen receptors. Afferent input from the telencephalic nucleus HVc (also known as the "high vocal center") is necessary for seasonal growth of the direct efferent target nuclei RA and area X. We asked here whether T-stimulated growth of HVc is sufficient to induce growth of its efferent nuclei. Intracerebral T implants were placed unilaterally near HVc or RA in photosensitive adult male white-crowned sparrows for one month. The T implant near HVc produced significant growth of the ipsilateral (but not contralateral) HVc, RA, and area X, and increased neuronal number in the ipsilateral HVc. The T implant near RA did not produce selective growth of ipsilateral RA, HVc, or area X. Intracerebral T implants did not elevate plasma T levels, nor did they stimulate growth of two peripheral androgen sensitive targets, the syrinx and the cloacal protuberance. These results suggest that seasonal growth of the adult song circuits results from T acting directly on HVc, which then stimulates the growth of RA and area X transynaptically.

Seasonal changes in the morphology of adult brain nuclei have been observed in representatives of every vertebrate class (1). The avian song control system has emerged as a leading model for studying seasonal plasticity in the adult brain. The nuclei that control song learning and production in birds are easily identified, and their connectivity is well established (Fig. 1; for review, see refs. 2 and 3). There is pronounced seasonal plasticity in the volume and neuronal attributes of nuclei in the song control system (reviewed by ref. 1). These seasonal changes in the song system are primarily regulated by changes in plasma levels of testosterone (T) that are correlated with changes in photoperiod (4–6). Afferent input from the telencephalic nucleus HVc is necessary for the seasonal growth of its efferent nuclei within the song control circuits (7). The present study was designed to test the hypothesis that seasonal-like growth of HVc is sufficient to induce growth of its efferent nuclei.

Growth of HVc in response to breeding season cues precedes that of its direct efferent target nuclei RA (robust nucleus of the archistriatum) and area X in song sparrows (*Melospiza melodia*) and Gambel's white-crowned sparrows (GWCS, *Zonotrichia leucophrys gambelii*) (8, 9). These observations led to the hypothesis that afferent input from HVc is required for the growth of RA and area X. Afferent input is important in the development and maintenance of diverse neural systems (e.g., refs. 10 and 11–14). In the birdsong system, depriving RA and area X of afferent input in juvenile zebra finches (*Taenopygia guttata*) prevents these nuclei from growing to their normal size in males (15, 16), and blocks the masculinization of these regions induced in females by early treatment with estradiol (17). If plasticity in adult song circuits depends on mechanisms similar to those used in juvenile development (1), then we predicted that depriving adult song nuclei of their afferent input would prevent their growth in response to seasonal-like cues. In support of this prediction, unilateral lesions of HVc in adult male GWCS completely blocked the growth of the ipsilateral (but not contralateral) RA and area X in response to systemic implants of T and exposure to a long day (LD) photoperiod (7). This result is particularly interesting in the case of RA because it has abundant androgen receptors (Fig. 1; reviewed by refs. 2 and 18), but does not grow in response to high T levels in the absence of afferent input from HVc. Given the necessity of afferent input for the growth of the song control circuits demonstrated in our previous study, we predicted that inducing growth of HVc would be sufficient to stimulate growth of its efferent targets as well. In the present study, we found that intracerebral implants of T placed unilaterally adjacent to HVc stimulated growth of the ipsilateral but not contralateral efferent nuclei.

Materials and Methods

Collection of Birds and Experimental Treatments. We captured 11 adult male GWCS in eastern Washington during their post-breeding season migration in September and October 1999. All birds were housed indoors in group aviaries for at least 12 wk on short days (SD, 8 h light) before the start of the experiment to ensure that their reproductive systems were regressed but sensitive to the stimulating effects of T (i.e., they were photosensitive). White-crowned sparrows kept on SD indefinitely maintain regressed testes, basal or nondetectable levels of circulating T, and regressed song control nuclei that are typical of the nonbreeding season (19–22). At the start of the study, each bird was placed in an individual cage and housed in visual and auditory contact with all of the other birds in the same treatment group in a single room.

We prepared T pellets for intracerebral implantation by using a modification of the method of Grisham *et al.* (23). We ground 0.1 g crystalline T (Steraloids, Wilton, NH) to a fine powder in a mortar. The T was combined with 0.03 g inert activated charcoal powder, used to visualize the implant, and 0.95 g uncured medical grade Silastic sealant, and this mixture was spread uniformly in a well that was formed between two stacks of coverslips glued to a glass slide. After the Silastic sealant cured overnight we used a blunt-tipped 18-gauge syringe needle to punch pellets, which were extruded with a wire. Each pellet contained about 7 μg T; we used this low dose to minimize diffusion of the T away from the implant site (see below). To implant one pellet in each bird's brain, we loaded it into the end of a 22-gauge blunted syringe needle, which was fixed to a stereotaxic plunger. We lowered the tip of the needle to the desired location in the brain of birds anesthetized with isoflurane and gently extruded the pellet by inserting a wire into the needle

NEUROSCIENCE

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: AR, androgen receptor; BDNF, brain-derived neurotrophic factor; CP, cloacal protuberance; GWCS, Gambel's white-crowned sparrow; LD, long day; RA, robust nucleus of the archistriatum; SD, short days; T, testosterone; nXIIts, the tracheosyringeal portion of the hypoglossal nucleus.

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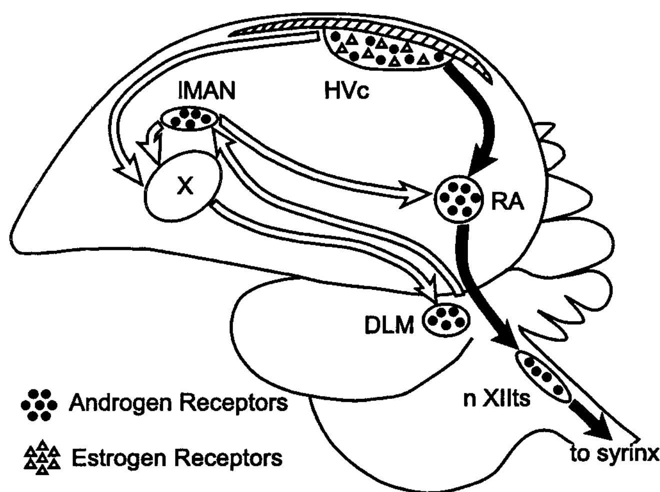

Fig. 1. Simplified schematic sagittal view of the avian song control system showing the distribution of steroid receptors. Black arrows connect nuclei in the main descending motor circuit, and white arrows connect nuclei in the anterior forebrain circuit. DLM, Dorsolateral nucleus of the medial thalamus; lMAN, lateral portion of the magnocellular nucleus of the anterior neostriatum; nXIIts, the tracheosyringeal portion of the hypoglossal nucleus; RA, the robust nucleus of the archistriatum; syrinx, vocal production organ; V, lateral ventricle; X, area X of the parolfactory lobe.

shaft; the wire was bent at the appropriate length to prevent it from extending too far out of the distal end of the shaft. The needle remained in place for 5 min after extruding the pellet and was then slowly removed. The scalp was sutured.

We implanted a T pellet adjacent to HVc on one side of the brain in five birds, alternating between the left and right sides in successive birds. The unimplanted side of the brain served as an internal control for the T implant. We directed the implants to the telencephalon lateral and ventral to the main corpus of HVc so as to avoid mechanical damage of the nucleus. Histological inspection of the brains later showed that the T pellets in different birds were within 0.1–1.1 mm (X ± SEM = 0.58 ± 0.38 mm) of the closest boundary of HVc.

Steroid hormones are lipophilic and therefore readily diffuse away from an implant site in the brain (e.g., ref. 24). If we observed growth of RA [which has abundant androgen receptors (ARs)] when T was implanted near HVc in the previous experiment, then it would be possible that RA grew directly in response to T that diffused from the implant site rather than indirectly from an effect of T on HVc. To test for this possibility, we implanted a T pellet adjacent to RA on one side of the brain in six other birds, using the same methods as for the HVc implants. The implants were located 0.25–1.0 mm (0.56 ± 0.32 mm) lateral of RA. The distance of implants from the target nucleus did not differ between the HVc and RA implant groups (t = 0.09, df = 8, P = 0.930).

Photoperiod can influence the growth of song nuclei by a mechanism that is independent of gonadal steroid hormones (reviewed in ref. 25). In a previous study of GWCS, for example, area X in castrated birds exposed to LD was intermediate in size between castrates exposed to SD and castrates exposed to SD + T (4). To ensure that any growth of song nuclei that occurred was due to the effects of the intracerebral T implants rather than to steroid-independent effects of photoperiod, we housed the birds on SD throughout the study.

Histology and Brain Morphometry. Four weeks after we implanted the T pellets, the birds were deeply anesthetized with methoxyflurane and perfused through the heart with heparinized saline followed by 10% neutral buffered formalin (NBF). Brains were postfixed in NBF for at least 2 wk, embedded in gelatin, cryoprotected in a 20% sucrose-NBF solution, and sectioned in the coronal plane at 50 μm on a freezing microtome. Every other section was mounted and stained with thionin. The Nissl-defined borders of song nuclei coincide with the borders as defined by other labels (reviewed by ref. 1).

We projected a magnified image (×46) of each mounted section that contained a song nucleus profile (100 μm sampling interval). We traced onto paper the Nissl-defined borders of HVc, RA, area X, and nXIIts (the tracheosyringeal portion of the hypoglossal nucleus) ipsilateral and contralateral to the T implants near HVc or RA. We used the criteria of DeVoogd *et al.* (26) to distinguish the tracheosyringeal from the lingual portion of the hypoglossal nucleus (see ref. 8). These tracings were scanned into a computer, and the cross-sectional area of each song nucleus profile was calculated by using IMAGE software (version 1.57, Wayne Rasband, National Institutes of Health, Bethesda, MD). We estimated the volume of each nucleus by using the formula for a cone frustum over each measured profile area (21). All brain measurements were made blind to treatment group.

We also measured neuronal attributes of HVc, including soma area, neuronal density, and neuronal number, as described below. In wild birds, neuronal number in HVc increases dramatically during the breeding season (1, 8, 27). We wanted to determine whether the intracerebral T implants near HVc similarly increased neuronal number. Neuronal number in RA, area X, and nXIIts do not change seasonally, and we therefore did not examine them in this study (8, 9).† There are pronounced seasonal changes in soma area and neuronal density in RA and area X (8, 9).† In HVc, soma area of neurons increases to a smaller extent during breeding, and neuronal density does not change (8, 9, 21). Similarly, soma area but not density of neurons in nXIIts changes seasonally (9).

We used a random systematic sampling scheme to measure soma size and neuron density in HVc. This procedure was described in detail by Tramontin *et al.* (28), who showed that, with 50-μm Nissl-stained sections, this scheme yielded estimates of neuron density and number that did not differ from those obtained by using a stereological optical disector procedure. Briefly, we captured a video image of HVc at ×195 magnification by using IMAGE. We outlined the borders of HVc onscreen, and overlaid it with a square grid 42 μm per side. By using a random number generator, we selected one of the first six columns, and every sixth column thereafter. For each selected column, we randomly selected one square. We counted and measured the soma area of all neurons in that square from a second digitized image of that field captured at ×1,950 magnification. In this way, we measured at least 150 neurons throughout the HVc of each bird that received a T implant near HVc, which required sampling 17–23 fields per individual. This sample size is sufficient to encompass all of the variance present in soma area and neuron density in HVc (28).

Hormone Assay and Measurement of Peripheral T Targets. T released by the intracerebral implants could have entered the circulatory system and been carried to other hormone-sensitive song nuclei, where it might have acted to stimulate their growth. To determine whether T entered the systemic circulation in significant levels, we measured plasma T concentrations and two peripheral T targets: the cloacal protuberance (CP), an androgen-sensitive secondary sex structure involved in sperm delivery; and the vocal production organ (i.e., the syrinx), the muscles of which have AR (29–31). If plasma T levels were significantly elevated, then we

†Thompson, C. K. & Brenowitz, E. A. (2002) *Soc. Neurosci. Abstr.* **28,** in press.

Table 1. Volumes (mm^3) of song control nuclei (X ± SEM) ipsilateral and contralateral to unilateral T implants near HVc or RA

Region	Implant site	Ipsilateral	Contralateral	t_{df}	*P*
HVc	HVc	0.505 ± 0.054	0.406 ± 0.063	10.23_4	**<0.0005**
	RA	0.569 ± 0.053	0.601 ± 0.079	-0.89_5	0.207
RA	HVc	0.186 ± 0.011	0.165 ± 0.012	4.05_4	**0.008**
	RA	0.179 ± 0.018	0.190 ± 0.057	-2.13_5	**0.043**
Area X	HVc	0.970 ± 0.103	0.828 ± 0.090	7.60_4	**0.001**
	RA	0.987 ± 0.096	0.981 ± 0.099	0.08_5	0.468
nXIIts	HVc	0.031 ± 0.001	0.028 ± 0.003	0.75_3	0.254
	RA	0.032 ± 0.003	0.029 ± 0.004	1.08_4	0.170

The *t* statistic, df (in subscript), and *P* level for each ipsilateral × contralateral comparison are indicated. Significant test results are shown in bold type.

might expect to observe growth of the CP and/or increased syringeal mass (e.g., refs. 9 and 30).

We measured plasma T levels for each bird at four time points: 14 and 4 days before they received T implants, and 4 and 30 days after the implants were delivered (on day 0). We collected 300 μl of whole blood into heparinized microcentrifuge tubes by puncturing the alar vein. Each blood sample was immediately centrifuged, and the plasma was removed and stored at −20°C until assay. We measured T in the plasma by RIA using the Coat-A-Count Total Testosterone kit (Diagnostic Products, Los Angeles). The use of this assay to measure plasma T has been validated for songbirds (32). The minimum detectable plasma T concentration was 0.1 ng/ml. Samples with undetectable T levels were treated as having concentrations at this detection limit for statistical analysis. We measured the samples in three different assay runs; the interassay coefficient of variation was 8%, and the intraassay coefficients of variation were 6.6%, 9.4%, and 5.4%.

CP length was measured on day 0 and again 30 days after the implants were delivered. The syrinx was dissected free of the tracheobroncheal junction immediately after perfusion, stored in neutral buffered formalin, and weighed. We compared the syringeal weights of our birds with those of male GWCS that had been housed on SD for 12 wk in the study of Tramontin *et al.* (9). The birds in the study of Tramontin *et al.* had basal plasma T levels and regressed song control systems; their syringeal weight was significantly less than that of males implanted with a systemic T pellet and housed on LD for either 7 or 20 days.

Song Behavior. We monitored all birds for the occurrence of song behavior on alternate days throughout the 30 days of the experiments. We observed birds with a videocamera and microphone for 1 h when the room lights first came on in the morning. "Dawn" is the time of day when song is produced most frequently by birds housed on LD in captivity and during the breeding season in the wild (i.e., the "dawn chorus"). Adult GWCS captured in the wild and housed in captivity on SD typically do not sing (e.g., ref. 9), whereas wild GWCS do sing occasionally outside the breeding season (e.g., ref. 21).

Statistical Analyses. One-tailed paired *t* tests were used to compare the volumes of each song nucleus, and neuronal attributes of HVc, ipsilateral and contralateral to the T implants. We used one-tailed tests because we tested the directional hypotheses that T implants near HVc or RA would cause growth of ipsilateral nuclear and neuronal attributes. There is no basis for predicting that these intracerebral implants would result in smaller song nuclei or smaller/fewer HVc neurons ipsilateral to the implants compared with the contralateral side of the brain.

We used a nonparametric Friedman repeated measures ANOVA to compare plasma T levels across the four time points sampled in each bird. Sampling time was the repeated measure.

A one-tailed paired *t* test was used to compare CP length in each bird at days 0 and 30. We used a Mann–Whitney rank sum test to compare syrinx weight between the birds in our study and the SD birds from the study of Tramontin *et al.* (9).

Results

T Implant near HVc. Intracerebral implants of T placed adjacent to HVc on one side of the brain induced significant growth of the ipsilateral HVc but not contralateral HVc (Table 1, Fig. 2). In addition, the efferent targets of HVc, RA, and area X grew on the implanted side but not on the contralateral side. The overall volume of all three nuclei was greater on the side of the brain ipsilateral to the T implant than on the contralateral: HVc (t = 10.23, df = 4, $P < 0.0005$); RA (t = 4.05, df = 4, P = 0.008); area X (t = 7.60, df = 4, P = 0.001). The volume of the ipsilateral HVc was not significantly correlated with the distance from the T implant in different birds (r = −0.675, P = 0.211). For each of these three nuclei, the volume contralateral to the T implant did not differ from that of male GWCS housed on SD without T implants in the study of Brenowitz and Lent (7) ($P \geq 0.13$, Student's *t* tests); this result indicates that there was no growth of the contralateral nuclei in the present study. nXIIts did not differ in volume between the two sides of the brain (t = 0.75, df = 3, P = 0.254).

T implanted adjacent to HVc increased neuronal number in the ipsilateral HVc by 37% relative to the contralateral HVc (Table 2; t = 2.94, df = 4, P = 0.02). Neither soma area (t = 0.91, df = 4, P = 0.206) nor neuronal density (t = 1.36, df = 4, P =

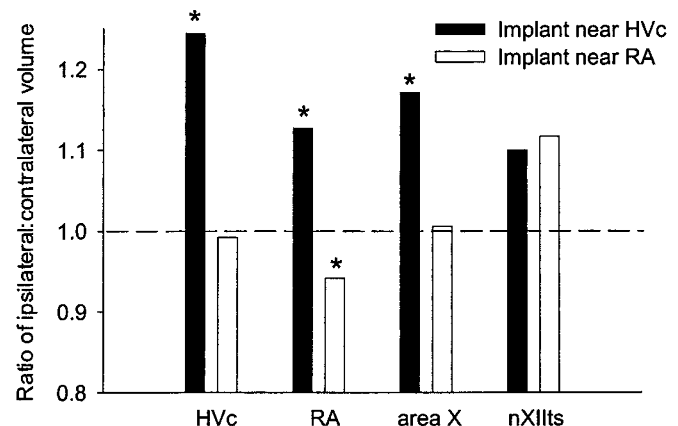

Fig. 2. The ratio of the mean volumes of song nuclei ipsilateral and contralateral to T implanted unilaterally near HVc or RA. The dashed horizontal line indicates a ratio of 1.0 expected if there was no difference in size between the ipsilateral and contralateral nuclei. *, Differences in volume between the ipsilateral and contralateral sides were significant (see Table 1).

NEUROSCIENCE

Table 2. Neuronal attributes of HVc ipsilateral and contralateral to T implanted unilaterally adjacent to HVc

	Ipsilateral	Contralateral
Soma area, μm^2	74.30 ± 7.57	72.82 ± 7.04
Density, $\times 10^4$ neurons/mm^3	14.23 ± 21.22	12.77 ± 11.83
Number, $\times 10^4$	7.78 ± 1.49	5.68 ± 0.87*

Values shown are means ± SEM. $n = 5$ birds for each side of the brain for each attribute. *, The difference in neuronal number between HVc ipsilateral and contralateral to the T implant was significant at $P = 0.021$, paired t test.

0.122) differed significantly between the ipsilateral and contralateral sides of HVc.

T Implant Near RA. Implants adjacent to RA failed to produce significant growth of any song nuclei (Table 1, Fig. 2). Nuclear volume did not differ between the ipsilateral and contralateral sides for HVc ($t = -0.89$, df = 5, $P = 0.207$), area X ($t = 0.08$, df = 5, $P = 0.468$), and nXIIts ($t = 1.08$, df = 4, $P = 0.170$). RA was actually somewhat larger contralateral to the implant and this difference was marginally significant ($t = -2.13$, df = 5, $P = 0.043$).

Hormone Assay and Measurement of Peripheral T Targets. Plasma T levels were not elevated by the intracerebral T implants (Table 3). There was no significant change in circulating T concentrations between the two preimplant and two postimplant sampling times ($\chi^2 = 3.00$, df = 3, $P = 0.392$).

Peripheral androgen-sensitive targets did not grow in response to the intracerebral T implants. CP length in the GWCS did not differ before (X ± SEM = 2.75 ± 0.21 mm) and after (2.89 ± 0.20 mm) they received the T implants ($t = -0.81$, df = 7, $P = 0.222$). Syrinx weight did not differ ($T = 44.0_{5,11}$, $P = 0.46$) between the birds in the present study (median, interquartile range = 130 mg, 121–164 mg) and the SD GWCS (132 mg, 125–367 mg) from the study of Tramontin *et al.* (9).

Song Behavior. None of the GWCS were observed to sing in either the HVc or RA-implanted groups. This result is consistent with our previous observation that adult GWCS captured in the wild and held in captivity on SD typically do not sing (e.g., 9).

Discussion

Transynaptic effects of gonadal steroids are known to influence the development of neural circuits (reviewed by ref. 12). To our knowledge, the current study presents a previously undocumented demonstration that steroids can also act transynaptically to stimulate seasonal-like growth of neural circuits in the adult brain. Seasonal growth is a common feature of adult vertebrate brains (reviewed by ref. 1), and the transynaptic action of hormones that we observed may be a general mechanism underlying this form of plasticity.

Table 3. Plasma T levels (ng/ml) at two time points before and two time points after birds received intracerebral T implants (at day 0)

Day −14	Day −4	Day +14	Day +30
0.10 (0.10–0.25)	0.10	0.10	0.10

Value shown for the first time point is median (interquartile range). For the latter three time points, T levels for all birds were below the minimum detection limit of the assay (0.1 ng/ml). Birds were sacrificed on day 30. Data for the HVc and RA T implant groups were combined. Plasma T levels did not vary significantly before and after the intracerebral T implants (Friedman repeated measures ANOVA, $P = 0.392$).

Previous research demonstrated that seasonal growth of the song circuits is primarily regulated by changes in plasma T levels (4–6). Lesions of HVc block the growth of its efferent targets RA and area X in GWCS exposed to breeding-like systemic levels of T and LD (7). Taken together with the present study, these results suggest that T acts directly on HVc, which in turn stimulates the growth of RA and area X via transynaptic effects. AR are present in both RA-projecting and area X-projecting neurons in HVc (33).

Several observations suggest that the growth of the ipsilateral RA and area X in the birds with T implants near HVc was due to a trophic effect of T directly on the ipsilateral HVc, rather than to diffusion of T through the brain or transport in the circulation to RA or area X: (*i*) T implanted near RA did not induce selective growth of the ipsilateral RA, HVc, or area X, as would be expected if T acted directly on RA or diffused through the brain to the ipsilateral HVc or area X; (*ii*) there was no growth of the contralateral song nuclei when T was implanted unilaterally near HVc or RA, as might be expected if T was transported to the other side of the brain at significant levels in the circulation; (*iii*) circulating T levels measured in the periphery were not elevated by the intracerebral implants; and (*iv*) peripheral androgen-sensitive targets, the CP and the syrinx, did not grow in response to intracerebral T.

Intracerebral T implants near HVc increased neuronal number in the ipsilateral (but not contralateral) HVc. This observation is analogous to the increase in HVc neuronal number that occurs in wild birds during the breeding season and in captive birds treated with systemic T (reviewed by ref. 1). The increase in HVc neuronal number may reflect enhanced recruitment and/or survival of new HVc neurons caused by the intracerebral T implants. Support for this suggestion comes from the study of Rasika *et al.* (34). They observed that treatment of adult female canaries with systemic T implants increased the number of new RA-projecting neurons in HVc. Furthermore, those authors showed that the action of T on neuronal recruitment/survival in HVc is mediated by brain-derived neurotrophic factor (BDNF; ref. 35). Systemic T treatment increased the levels of BDNF protein in female canary HVc, infusion of BDNF into HVc tripled the number of new neurons, and infusion of a neutralizing antibody against BDNF prevented the T-induced increase in new HVc neurons. A similar interaction between T and BDNF may have influenced the increase in HVc neuronal number in our study; BDNF mRNA is expressed by HVc neurons in adult male GWCS (A.-M. Wissman, E. Chartoff, D. Dorsa, and E.A.B., unpublished observation).

The magnitude of the volumetric changes in HVc, RA, and area X caused by the intracerebral T implants was less than that seen in wild birds during the breeding season (e.g., refs. 8 and 36) and captive birds treated with systemic implants that induced breeding-typical plasma T levels (e.g., refs. 4 and 9). Neuronal number in HVc increased by 37% in our study, whereas systemic T implants in GWCS increased neuronal number by 67% (9). Soma area of ipsilateral HVc neurons did not increase significantly in our study, whereas there are small but significant increases in soma area in breeding-condition birds as discussed above. These differences probably reflect the small dose of T used in the present study (≈7 μg per implant). These intracerebral implants likely yielded local concentrations of T in HVc that were much lower than those associated with breeding-typical plasma levels of 4–10 ng/ml in wild birds (37), or that are released by systemic implants that contain ≈12 mg of T and produce plasma T levels in captive birds in the physiological breeding range (e.g., ref. 9).

It is notable that RA did not grow in response to adjacent T implants, even though these implants were the same distance from RA as from HVc, and cells in RA have high levels of AR (e.g., ref. 38). This result is consistent with the observation that

RA did not grow in response to systemic T implants that produced breeding-typical plasma T levels if the ipsilateral HVc was lesioned (7). There are several possible explanations for these observations: (*i*) Full seasonal-like growth of RA may require an interaction between transynaptic influences from HVc and the direct action of T on RA. (*ii*) AR expression in RA neurons may be down-regulated in nonbreeding-condition birds (e.g., ref. 39) and require trophic input from HVc to be up-regulated. Decreased AR levels would make RA less sensitive to the stimulatory effects of T. In addition, there is a somewhat lower proportion of T-accumulating cells in RA (≈47%) than in HVc (57%) in canaries (40). If a similar difference exists in GWCS, then RA may be less sensitive to T than HVc. (*iii*) AR in RA neurons may not play a role in seasonal morphological growth of this nucleus, but rather be involved in activational aspects of androgen function such as synaptic transmission (e.g., refs. 41 and 42). For example, there may be functionally different isoforms of the AR in HVc and RA neurons, as observed in other systems (e.g., refs. 43–45). There also may be differences between HVc and RA neurons in the ability of ligand-bound androgen receptors to activate genes that are involved in morphological plasticity, such as those that encode cytoskeletal proteins, or neurotrophins and their receptors (e.g., see refs. 46–48).

The specific nature of the T-induced trophic support provided by HVc to its efferent targets is not yet known. The release of neurotransmitter molecules across chemical synapses may have trophic effects on postsynaptic neurons (14, 49–53). Synaptic input from HVc may have this type of trophic effect on neurons in RA and area X. Alternatively, or in addition, presynaptic terminals from HVc may release neurotrophins that stimulate growth of postsynaptic neurons. Neurotrophins can be transported anterogradely and taken up by postsynaptic neurons (54, 55). As discussed above, neurotrophins are present in the song system (see also ref. 56) and influence the juvenile development of the song circuits (57–59). The effects of intracerebral T implants near HVc on the ipsilateral HVc and its efferent targets in our study may have also been mediated by the action of neurotrophins. The possible roles of chemical neurotransmission and neurotrophins in the trophic support provided by HVc to its efferent nuclei are discussed further in Brenowitz and Lent (7).

nXIIts did not grow in response to intracerebral T implants adjacent to either the ipsilateral HVc or RA. This result is consistent with the observation that nXIIts did grow in response to LD + systemic T implants even when the ipsilateral HVc was lesioned (7). Seasonal-like growth of nXIIts thus appears to occur independently of HVc input to the motor circuit. There are several possible explanations for these observations: (*i*) Neurons in nXIIts have AR (60) and may respond directly to high plasma T levels. (*ii*) T may directly stimulate the syringeal muscles, which have AR (29–31). The motor neurons in nXIIts innervate these muscles and might derive retrograde trophic support from them, as observed in the spinal nucleus of the bulbocavernosus of rats (61, 62) and the laryngeal motor nucleus of *Xenopus* (63). (*iii*) Neurons in nXIIts receive afferent input from the dorsomedial portion of the intercollicular nucleus (ICo), which has AR (e.g., ref. 64). T may act on ICo neurons, which could then have trophic effects on postsynaptic nXIIts neurons.

Intracerebral steroid implants have been used to localize the sites of hormone effects on the development of brain regions (e.g., ref. 23), and adult sexual and aggressive behavior (e.g., refs. 24 and 65–67). Our study demonstrates that this method can also be used to identify the site of action of steroid hormones in mediating seasonal growth of neural circuits in the adult brain. Given the prevalence of seasonal changes in vertebrate brains, the use of intracerebral implants of hormones and their antagonists may be of broad applicability in studies of this form of adult plasticity.

We thank David Perkel, Kira Wennstrom, Anne-Marie Wissman, and anonymous referees for comments on the manuscript. Bill Grisham and Greta Matthews kindly provided technical advice on fabrication of intracerebral implants. This research was supported by National Institutes of Health Grant MH53032 (to E.A.B.), the Virginia Merrill Bloedel Hearing Research Center, and University of Washington Research Core Center National Institutes of Health Grant P30 DC04661.

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