Photoperiod and Testosterone Independently Affect Vocal Control Region Volumes in Adolescent Male Songbirds

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ABSTRACT: Previously, we found that, unlike adults, adolescent male dark-eyed juncos (*Junco hyemalis*) maintained large Area X volumes despite having low plasma testosterone concentrations. Other studies indicate that photoperiod may act independently of testosterone to modulate vocal control region (VCR) volumes in adult songbirds. In the present study, we investigated the effects of testosterone and photoperiod on the volumes of four VCRs in adolescent male juncos. To test the hypothesis that VCR volumes in these males are testosterone independent, we treated birds exposed to short days with testosterone and later compared their VCR volumes with those of birds exposed to short days without testosterone. To examine whether photoperiod alone could affect VCR volumes independent of testosterone, we measured these volumes in photorefractory birds exposed to long photoperiod without testosterone. Administering testosterone induced singing, yet increased the volume of only one VCR, the robust nucleus of the anterior neostriatum (RA). In contrast, long photoperiod increased several VCR volumes (Area X, higher vocal center, and RA) despite low testosterone levels, but did not induce singing. Our results suggest a limited role for testosterone, but an important role for photoperiod, in controlling VCR volumes in adolescent male juncos. In addition, the results demonstrate that singing behavior can be induced in adolescent males without a concomitant increase in most VCR volumes. J Neurobiol 36: 550–558 1998

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The brain regions [vocal control regions (VCRs)] that control song learning and production in songbirds form an interconnected vocal control system that exhibits neuronal plasticity throughout adulthood in many species (Nottebohm et al., 1976, 1986; Smith, 1996; Brenowitz et al., 1996b; Gulledge and Deviche, 1997) (Fig. 1). Changes in VCR size result from alterations in cell number and/or cell size (Brenowitz et al., 1991; Smith, et al., 1995). In seasonally breeding adult songbirds, VCR volumes are large during the breeding (singing) season, when days are long and plasma testosterone (T) concentrations are high, and smaller after the breeding season, when both day length and circulating T levels decrease (Smith, 1996; Brenowitz et al., 1996b; Gulledge and Deviche, 1997). This seasonal pattern can be simulated in captivity by exposing adult males to either long photoperiod or exogenous T, and comparing their VCR volumes to birds exposed to short photoperiod and low plasma T levels (Nottebohm, 1981; Brenowitz et al., 1991; Smith et al., 1995).

The effects of T are presumably mediated by intracellular androgen receptors (ARs). These receptors are located in brain nuclei that control song production [(HVc), also known as the higher vocal

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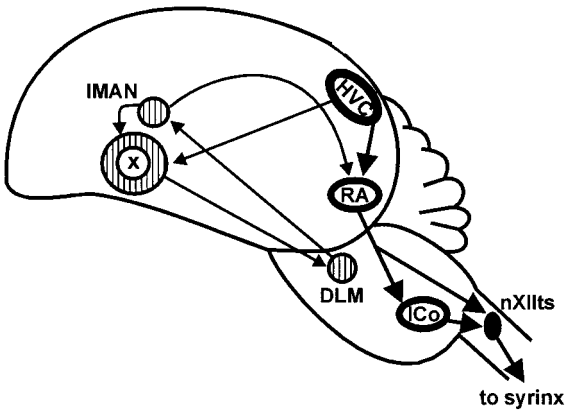

Figure 1 Diagram of the avian vocal control system. Regions in black form the motor pathway and hatched regions form the anterior forebrain pathway. DLM = dorsolateral n. of the medial anterior thalamus; Ico = n. intercollicularis; lMAN = lateral n. of the anterior neostriatum; nXIIts = tracheosyringeal portion of the hypoglossal nucleus; RA = robust n. of the archistriatum; X = Area X.

center; robust nucleus of the archistriatum (RA); nucleus intercollicularis (ICo); tracheosyringeal portion of the hypoglossal nucleus (nXIIts)], and form the ''motor pathway'' (Arnold et al., 1976; Smith et al., 1996) (Fig. 1). They are also found in one region [lateral portion of the magnocellular nucleus of the anterior neostriatum (lMAN)] that is part of a second pathway (anterior forebrain pathway) controlling song learning (Bottjer et al., 1984; Smith et al., 1996). Another anterior forebrain region, Area X, is involved in song learning and changes size seasonally. Area X receives projections from HVc but does not contain ARs. The effects of T on this region therefore may be mediated indirectly via projections from HVc (Arnold, 1980; Gahr, 1990). The lMAN also sends projections to Area X and may participate in the effects of T on this region (Nixdorf-Bergweiler et al., 1995; Vates and Nottebohm, 1995).

Androgen regulation of VCR volumes may be age and region dependent (Gulledge and Deviche, 1997). Castrating adult male dark-eyed juncos (*Junco hyemalis*) during the reproductive season causes both HVc and Area X to shrink compared to T-treated castrated males. In intact males, HVc volume increases from early adolescence (2- to 3-month-old birds) to adulthood in parallel with plasma T. These results suggest that HVc volume is affected by plasma T levels in adolescence, as well as in adulthood. In contrast, Area X in adolescent males is the same size as in breeding adult males, even though plasma androgen levels are low in adolescence and high during the breeding season (Gulledge and Deviche, 1997). We speculated that Area X volume maintenance is independent of elevated circulating T levels in adolescence, but not in adulthood. Therefore, artificially elevating T in adolescent males should not affect Area X volumes, but may affect HVc volumes.

Testosterone is not the sole modulator of VCR volume; photoperiod may exert effects on VCRs in adults that are independent of its temporal relationship with plasma T. For example, in castrated adult male Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), long photoperiod had small stimulatory effects on HVc volume and neural size in RA that were independent of, but also smaller in magnitude than those of, T (Smith et al., 1997a). Similar T-independent effects of photoperiod on region volume were detected in HVc, RA, and Area X of adult male American tree sparrows (*Spizella arborea*) (Bernard et al., 1997). In nature, the vernal seasonal increase in circulating T concentration follows increasing photoperiod (Wingfield and Farner, 1978). Seasonal increases in VCR volumes and singing rates may result from a cumulative effect of long photoperiod and elevated T levels (Nowicki and Ball, 1989; Smith et al., 1997a; Bernard et al., 1997).

In addition to photoperiod, the photoperiodic condition of the animal may influence the response of VCR volumes to long photoperiod or elevated T levels (Bernard and Ball, 1995, 1997). Songbirds hatch in a photorefractory condition, meaning that their reproductive systems are not responsive to long photoperiods (Nicholls et al., 1988). Continuous exposure to long photoperiod maintains photorefractoriness; only exposure to short days, as in autumn and winter, allows those birds to become photosensitive or responsive to long days. At the end of the summer breeding season, adults become photorefractory again and must be exposed to short days to regain photosensitivity. Photorefractory adult male European starlings (*Sturnus vulgaris*) exposed to long photoperiod did not exhibit HVc volume growth in response to T treatment, whereas photosensitive birds receiving the same T treatment did have increased HVc volumes (Bernard and Ball, 1997). In contrast, castrated, photosensitive adult birds exposed to long photoperiod had VCR volumes that increased both dependently and independently of elevated T (Smith et al., 1997a). Although birds in both studies were exposed to long photoperiod, they were in different photoperiodic conditions (photorefractory vs. photosensitive), which may

explain why VCR volumes were large in one group exposed to long days (photosensitive) (Smith et al., 1997a), but not in the other (photorefractory) (Bernard and Ball, 1997). Together, these two studies indicate that birds may need to be photosensitive for long photoperiod or T to increase VCR volumes.

Captive adolescent juncos held on short days become photosensitive by mid-November (Gulledge and Deviche, unpublished data), and in the wild, respond to increasing photoperiod in midspring by secreting gonadal T and producing crystallized song. Measuring the effects of long photoperiod in photorefractory adolescents with low T levels should reveal whether long photoperiod alone can influence VCR volumes independently of photosensitivity or T. In addition, augmenting T in adolescent males exposed to short days should reveal whether T alone influences VCR volumes at this age as it does in adults. To test these predictions, we experimentally separated the effects of photoperiod/photoperiodic condition and plasma T on VCR volumes in adolescent male juncos. We compared VCR volumes of control birds (SD-C), which were short-day photosensitive and had low plasma T levels, with those of short-day photosensitive juncos with experimentally elevated plasma T levels (SD-T) and of long-day photorefractory birds with low T levels (LD).

MATERIALS AND METHODS

Animal Collection and Handling

Adolescent male juncos that hatched in June or July were collected near Fairbanks, Alaska (65°N, 147°W), using baited Potter traps in mid-September of 1996 (photoperiod approximately 12:12 h light/dark cycle). These birds were not singing, and were approximately 2–3 months old. Adult male juncos had ceased singing by that time (Gulledge and Deviche, unpublished observations), so the adolescents had been exposed to the normal duration of song tutoring at the time of capture. Adolescent males could be differentiated from adults based on plumage, eye color, and amount of skull pneumatization (Pyle et al., 1987). All necessary permits were obtained prior to bird collection. Males were kept in individual cages and received food and water *ad libitum.* The experimental design is represented schematically in Figure 2. Birds were initially exposed to a photoperiod (13:11 h light/dark cycle) similar to that of September in Fairbanks. On September 23, they were randomly divided into three groups. Two groups were exposed to short photoperiod (8:16 h light/dark cycle): SD-T birds ($n = 8$) received T-filled subcutaneous Silastic capsules (3 cm; 1.45-mm internal diameter, 1.93-mm outer diameter; Konigsberg

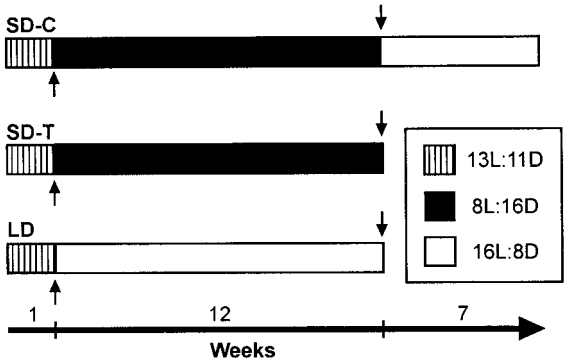

Figure 2 Schematic of the experimental design. Hatched bars indicate photoperiod of 13:11 h light/dark cycle; dark bars 8:16 h light/dark cycle; white bars 16:8 h light/dark cycle. ↑ = September 23, blood sample taken, capsule implantation. ↓ = December 13, blood sample taken, perfusion (except six extra SD-C birds). Numbers along the lower arrow indicate time in weeks for each photoperiod regime.

Instruments, Pasadena, CA); SD-C birds ($n = 14$) received empty capsules. The third group (LD; $n = 8$) also received empty Silastic capsules but was exposed to long photoperiod (16:8 h light/dark cycle) to maintain photorefractoriness. Blood samples (approximately 250 μL) were collected from a wing vein on September 23, 1996, and again on December 13, 1996. Samples were kept on ice until processed later the same day, at which time they were centrifuged and plasma was collected and stored at −20°C until assayed for T concentrations. On December 13, 1996, six SD-C birds were transferred to long photoperiod (16:8 h light/dark cycle) to determine whether they had gained photosensitivity, and thus, whether all SD birds were photosensitive by that time. Only photosensitive birds would respond to long photoperiod exposure by singing and rapidly developing their reproductive systems (Nicholls et al., 1988), so the photostimulated SD-C birds were monitored for singing behavior daily and widths of their cloacal protuberances (CPs) were measured after 6 weeks to assess reproductive system activity (Schwabl and Farner, 1989; Deviche, 1992). Typically, undeveloped junco CPs are 4 mm wide or smaller (Gulledge and Deviche, unpublished observations). Seven weeks after the extra SD-C birds were transferred to long photoperiod, they were euthanized and their testes were weighed.

Brain Processing

On December 13, 1996, all groups except the six extra SD-C birds received an intramuscular (i.m.) overdose of anesthetic (ketamine/xylacine), then an intracardial injection of heparin [0.3 mL; 1000 IU/mL in 0.1 *M* phosphate buffer (PB) solution], followed by room-temperature 0.1 *M* PB (20 mL) and cold 4% paraformaldehyde

solution in 0.1 *M* PB (25 mL). After the perfusion, testes were removed and weighed. The brains were postfixed in 4% paraformaldehyde solution overnight, dissected from skulls, and transferred to PB containing 0.1% Na azide for 2 days. Brains were then transferred to 30% sucrose solution in PB with Na azide for 4 days, after which they were blotted dry and weighed. Brains were coated with embedding matrix (M-1; Lipshaw), frozen in powdered dry ice, and stored at −70°C until processed further. They were coronally sectioned (50 μm) onto gelatin-coated slides in a cryostat at −15°C. The sections were dessicated overnight, then Nissl-stained with thionin.

VCR Volume Measurement

Vocal control regions (Area X, lateral and medial MAN, HVc, and RA) and a control region not involved in vocal behavior control, the nucleus rotundus (Rt), were identified on sections using the canary stereotaxic atlas (Stokes et al., 1974; Nottebohm et al., 1976). The ''inclusive'' boundaries of HVc were used to measure that region (Kirn et al., 1989). Lateral and medial MAN were measured together because the boundary between them was often difficult to distinguish. Regions were measured using the M1 MCID image analysis system (Imaging Research, St. Catherine, Canada), which calculates region volume by multiplying section thickness by region area. Areas were measured by using a computer mouse to trace the borders of each region projected on a monitor. Alternate sections were measured (left and right sides separately), the volumes of all measured sections in a brain were totaled and doubled, and values obtained for the left and right hemispheres were summed for each region. Previous studies indicate that volumes from right and left sides are not different (Gulledge and Deviche, unpublished data). Telencephalon width was also measured as a control for overall brain size. For this, all brain sections with the anterior commissure (CoA) present (usually three) were measured at the widest point, and the widths were averaged for each brain. Right and left hemispheres were measured separately, then summed. All data were collected without knowledge of bird identity. All data sets except MAN volume met analysis of variance (ANOVA) assumptions of normality and equal variance. Therefore, data for MAN were analyzed with a Kruskal–Wallis one-way ANOVA on ranks, and all other data were analyzed separately using one-way ANOVAs followed by Student–Newman–Kuels multiple comparisons tests when the ANOVAs indicated significant differences across groups. Data are presented as means ± standard errors, except data for MAN, which are presented as medians ± 0.5 interquartile intervals.

Testosterone Measurement

Plasma samples were assayed for T by direct radioimmunoassay (RIA) using a Coat-A-Count Total Testosterone kit (Diagnostic Products Corporation, Los Angeles, CA). This RIA is both sensitive (lower detection limit: 10 pg/tube) and specific (cross-reactivity: 3% with dihydrotestosterone, 0.02% with estradiol). Briefly, a standard curve was made in triplicate by adding known concentrations of T to tubes coated with antibody for T. Plasma samples (15–50 μL) were added in duplicate to antibody-coated tubes, and [125I]T (51,000 cpm/tube) was added to all tubes. Total counts (TC) and nonspecific binding (NSB) tubes (tubes containing only [125I]T) were included to determine the total radioactivity added to samples and the amount of NSB to the tubes, respectively. All tubes were incubated for 3 h at 37°C, contents of all but TC tubes were aspirated, and radioactivity was measured in a gamma counter for 2 min/tube. Final concentrations were corrected for amount of plasma added. The intra-assay coefficient of variation was 3.4%.

RESULTS

In September, all birds had undetectable plasma T concentrations. In December, only birds with T implants had detectable T levels (23.6 ± 2.0 ng/mL; high end of physiological range) (Gulledge and Deviche, unpublished observations). Most birds had undeveloped testes (all <5 mg, paired weight), although two SD-T birds had slightly developed testes (largest was 90 mg, paired weight). The SD-T birds began to sing about 2 weeks after receiving T implants. In December, the six photostimulated SD-C juncos responded to exposure to long photoperiod with singing and had enlarged CPs (5.5 ± 0.1 mm) and testes (150 ± 13 mg, paired weight) after 7 weeks of photostimulation, indicating that all SD birds were photosensitive at the end of the study. The LD birds never sang, and had undeveloped testes and small CPs (3.6 ± 0.1 mm) and undetectable plasma T concentrations at the end of the study, demonstrating that they remained photorefractory throughout the experiment.

Statistically significant differences among groups were detected in volumes of all VCRs measured except MAN (Fig. 3 and Table 1). LD birds had 37% larger Area X volumes than both SD groups, which did not differ from each other. LD birds had 40% larger HVc volumes than SD-C birds, but HVc volumes in SD-T birds did not differ from those of either LD or SD-C birds. Both LD and SD-T groups had larger RA volumes (31% and 37% larger, respectively) than measured in SD-C birds. Control measures (Rt volume, brain weight, and telencephalon width) did not differ among groups (Table 1), indicating that differences in VCR volumes were specific to the vocal control system and were not due to changes in overall brain size.

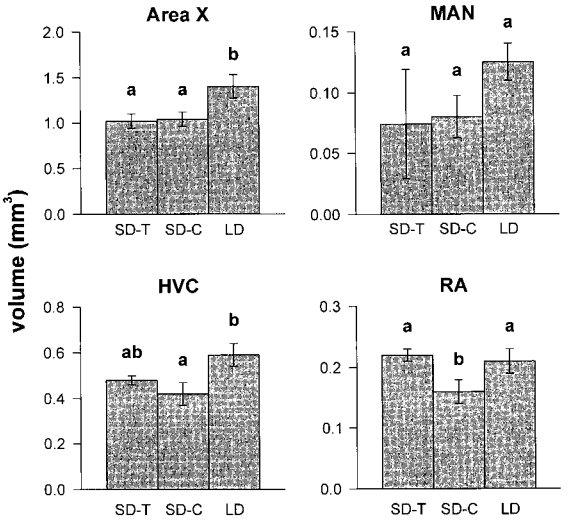

Figure 3 Characteristics (means ± SE, except for MAN, which is the median ± 0.5 interquartile interval) of vocal control regions (Area X, MAN, HVc, and RA) in adolescent male dark-eyed juncos exposed to short days with (SD-T) or without (SD-C) testosterone treatment (both photosensitive) or exposed to long days without testosterone treatment (LD; photorefractory). Differing superscripts indicate significant differences ($p < .05$) among treatments for a particular region.

DISCUSSION

This study indicates that T and long photoperiod have separate effects on VCR volumes in adolescent male juncos and that at this age, effects of T may be more limited than those of photoperiod.

Photoperiod Effects

Effects of long photoperiod on VCR volumes have often been assumed to be indirect, resulting from a stimulation of gonadal androgen secretion (Nottebohm, 1981; Brenowitz et al., 1991). When the effects of T and photoperiod are isolated experimentally, however, VCR volumes increase in response to long photoperiod alone in photosensitive adult white-crowned sparrows (Smith et al. 1997a). Bernard and Ball (1997) suggested that photoperiodic condition, rather than photoperiod per se, modulates the magnitude of VCR volume changes in response to T. They found that HVc volumes increase in response to T in photosensitive birds, but not in photorefractory birds. One implication of this finding is that photorefractoriness prevents the stimulatory effects of both long photoperiod and elevated T on HVc growth. Neither T nor photosensitivity, however, explains the stimulatory effects of long photoperiod that were observed in the present study. Indeed, photorefractory males exposed to long photoperiod (LD) in the fall had larger VCR (Area X, HVc, RA) volumes than photosensitive males exposed to short photoperiod (SD-C), and the two groups of birds had uniformly low circulating T levels. The stimulatory effects of LD on VCR volumes may involve melatonin, the secretion of which varies with photoperiod (Binkley, 1990). No role for melatonin has been identified in the vocal control system, but VCRs contain binding sites for this hormone, and densities of these sites are higher in HVc, RA, and Area X of male than of (nonsinging) female house sparrows (*Passer domesticus*) (Whitfield-Rucker and Cassone, 1996). Furthermore, melatonin receptor densities are unaffected by castration in house sparrows (Whitfield-Rucker and Cassone, 1996). Melatonin may therefore regulate T-independent influences of long photoperiod on VCR volumes.

Table 1 Measurements of Region Volumes and Overall Brain Size

Measure	SD-T	SD-C	LD	ANOVA
Area X (mm^3)	1.02 ± 0.8[a,†]	1.04 ± 0.08[a,†]	1.40 ± 0.13[b]	.026
MAN (mm^3)	0.07 ± 0.04[†]	0.08 ± 0.02[†]	0.13 ± 0.02	.124
HVc (mm^3)	0.48 ± 0.02[a,b,†]	0.42 ± 0.05[a]	0.59 ± 0.05[b]	.044
RA (mm^3)	0.22 ± 0.01[a]	0.16 ± 0.02[b]	0.21 ± 0.02[a]	.022
Rt (mm^3)	1.38 ± 0.07	1.58 ± 0.07	1.47 ± 0.07	.156
Brain weight (mg)	737 ± 19	781 ± 76	760 ± 21	.268
Telencephalon width (mm)	12.7 ± 0.1	13 ± 0.2	13.1 ± 0.2	.183
n	8	8	8	

Characteristics (means ± SE, except for MAN, which is the median ± 0.5 interquartile interval) of vocal control regions (Area X, MAN, HVc, and RA) and one nonvocal control region (Rt), as well as total brain size, in adolescent male dark-eyed juncos exposed to short days with (SD-T) or without (SD-C) testosterone treatment (both photosensitive) or exposed to long days without testosterone treatment (LD; photorefractory).

[†] $n = 7$.

[a,b] Differing superscripts indicate significant differences ($p < .05$) within a given row.

Testosterone Effects

Although RA, HVc, and MAN contain ARs (Arnold et al., 1976; Smith et al., 1996), only RA volume increased in response to T administration to adolescent males. A lack of response of MAN to T administration in this study is consistent with the fact that this region volume does not vary seasonally in free-living adult male juncos (Gulledge and Deviche, 1997). In adult male juncos, however, HVc volume changes seasonally. It shrinks following castration and is maintained at precastration levels by T administration in gonadectomized adult males, indicating that maintenance of this region's volume in adult males is T dependent (Gulledge and Deviche, 1997). One possible explanation for the lack of HVc volume increase in response to T administration in this study is that sensitivity of this region volume to plasma androgen concentrations develops after adolescence. As such, the increase in HVc volume measured between adolescence and adulthood in an earlier study on wild-caught juncos (Gulledge and Deviche, 1997) may have resulted from exposure to long vernal photoperiod rather than to increasing plasma T levels.

SD-T birds were photosensitive at the end of the experiment, but we do not know when they became photosensitive. Previous studies suggested that adolescent male juncos held on SD become photosensitive in early to mid-November (Gulledge and Deviche; Crain and Deviche, unpublished data). Bernard and Ball (1997) determined that T administration does not increase HVc volumes of photorefractory adult European starlings. If T treatment increases VCR volumes only in photosensitive birds, a second possible explanation for the present results is that HVc volumes did not differ between SD-T and SD-C birds because subjects were photosensitive only for a short time (2–4 weeks). The volume of RA, however, clearly increased in response to T administration. Thus, either T-induced growth of RA occurs in photorefractory males or T stimulation of RA volume takes place only in photosensitive birds, but occurs at a faster rate or earlier than HVc growth after the onset of photosensitivity. It should be noted that photorefractoriness did not inhibit photoperiod-induced growth of HVc in the LD birds. Photorefractoriness also did not prevent T induction of singing, as the SD-T birds were singing within 2 weeks of T treatment when they presumably were still photorefractory.

In adult male juncos, Area X volume decreases after castration, but is maintained at precastration levels in T-treated castrates, demonstrating that it is androgen dependent. In contrast, Area X volume in adolescent male juncos is maintained at breeding adult size despite the fact that adolescents have very low plasma T concentrations (Gulledge and Deviche, 1997). Apparently, then, T dependence of Area X volume develops after adolescence. The absence of ARs in Area X suggests that effects of T on this region in adults are mediated by projections from one or several AR-containing brain areas such as HVc and/or MAN (Gahr et al., 1996; Smith et al., 1996), the only AR-containing regions known to project to Area X (Nottebohm et al., 1976; Nixdorf-Bergweiler et al., 1995; Vates and Nottebohm, 1995). Neither HVc nor MAN responded to T administration in adolescent SD-T males, although HVc volume maintenance is T dependent in adult males (Gulledge and Deviche, 1997). Thus, the postadolescent development of T sensitivity of Area X may result from age-related changes in MAN and/or HVc.

Functional Considerations

The present data raise the question of the functional significance of VCR volume plasticity. Typically, plasma T concentrations, singing behavior, and VCR volumes are positively correlated in seasonal species (Nottebohm, 1980, 1981; Nottebohm et al., 1976; Brenowitz et al., 1991), and researchers tend to associate large VCRs with the ability to sing. In the present study, however, birds with relatively small VCRs (SD-T) sang, whereas the birds with the largest VCRs (LD) did not. Because elevated plasma T appears to be required for full song production (Arnold, 1975; Marler et al., 1988), the lack of singing by LD birds probably resulted from a lack of activation by T, despite increased VCR volumes. By contrast, the occurrence of song in the birds with smaller VCRs is not consistent with the idea that song expression directly depends on enlarged VCR volumes. SD-T birds sang even though the volumes of most of their VCRs did not differ from those of nonsinging SD-C birds.

The pattern of T effects on RA, Area X, and MAN volumes is consistent with current views on the functions of these regions in adults. RA is necessary for song production, and its volume was increased in birds induced to sing with T administration. Area X and MAN are necessary for song learning, but lesioning them after song crystallization does not immediately disrupt song expression (Scharff and Nottebohm, 1991; Sohrabji et al., 1990; Bottjer et al., 1984). A recent report that the immediate early gene *zenk* is induced in Area X

and lMAN during singing in deafened canaries (Jarvis and Nottebohm, 1997), however, indicates that these regions may play a role in song expression. This putative role may not depend on region size-enhancing effects of T, however, as Area X and MAN were not enlarged in the juncos induced to sing with T in this study.

The HVc plays an essential role in song production (Nottebohm et al., 1976; Yu and Margoliash, 1996), and we might expect it to be larger in singing than in nonsinging birds, which was not the case in this study. Recent evidence, however, suggests that changes in HVc volume may relate to aspects of song other than whether song is produced. For example, Smith et al. (1997b) found that male song sparrows sing during the fall, even though their plasma T levels are lower and their HVc volumes smaller than during the breeding season. Song structure, however, is more variable in the fall than during the breeding season, leading the authors to postulate that seasonal changes in HVc sizes relate to song complexity or stereotypy rather than just production. T administration to female canaries induces singing (Nottebohm, 1980). The VCRs of T-treated females, however, do not become as large as those of males, and T-induced female songs are not as complex as those of conspecific male songs (Nottebohm, 1980). Finally, results of Brenowitz et al. (1996a) suggested that female rufous and white wrens (*Thryothorus rufalbus*) may have smaller song repertoires than conspecific males because of their smaller HVc sizes. Junco songs are relatively simple, generally consisting of a trill composed of one rapidly repeated syllable (Konishi, 1964; Titus et al., 1997; Williams and MacRoberts, 1977). Smith et al. (1997b) noted that long trills may require many neurons to coordinate the rapid succession of syllables without fatiguing, and suggested that a smaller HVc may lead to trills of shorter duration. Songs produced by SD-T males resembled those expressed by intact free-living males, but we cannot exclude the possibility that songs of T-treated males differed from normal songs structurally or in their overall duration.

Other experiments on the effects of T during vocal control system development have produced differing results, depending on the species and the stage of development when T was administered. Bottjer and Hewer (1992) found that antiandrogen treatment disrupted vocal learning and reduced volumes of Area X and lMAN, but not HVc or RA, in castrated juvenile zebra finches (*Poephila guttata*). These results imply that maintenance of Area X and lMAN volumes depends on nongonadal T and that HVc and RA volumes are T insensitive. Differences with our results may be due either to species (zebra finches are critical-period learners that breed opportunistically whereas juncos are open-ended learners that breed seasonally) or to age (the zebra finches were only 20 days old at the beginning of treatments) differences. When zebra finches receive exogenous T at a similar age, their vocal learning is also impaired (Korsia and Bottjer, 1991), suggesting that inappropriate circulating levels of the steroid during sensitive periods alter normal vocal behavior development. Normally, peaks in circulating T concentrations are associated with closing of song learning periods both during development and during song modification in open-ended learners (Marler et al., 1987, 1988; Nottebohm et al., 1986). Premature song crystallization is the primary disruptive effect of T given too early in species, such as juncos, with a long song storage phase (Whaling et al., 1995). Prolonged T administration to yearling male juncos decreased singing rates without apparently affecting song structure, compared to birds induced to sing by photostimulation (Titus et al., 1997). These authors administered T just as plastic song was beginning, a later developmental stage than used in the current experiment. Our SD-T birds began singing within 2 weeks of T exposure, and like Titus et al. (1997), we did not detect any noticeable abnormality in song structure. Future studies that include time points at several stages of development through the first year of life may further elucidate the role(s) of T in modulating both VCR and song structure.

Conclusions

Three VCRs (Area X, HVc, and RA) were larger in photorefractory birds with low T (LD birds) than in photosensitive birds with low T (SD-C birds), but T administration to photosensitive birds (SD-T) increased only RA volume. These results support the hypothesis that control of Area X volumes is T-independent in adolescents, and suggest that long photoperiod may increase VCR volumes in adolescents by a mechanism that is independent of elevated plasma T and photoperiodic condition. In addition, we were able to dissociate T-induced singing from enlarged HVc volume, raising the issue of how closely changes in HVc volume correspond to the motor production of song.

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