Seasonal Changes in Testosterone, Neural Attributes of Song Control Nuclei, and Song Structure in Wild Songbirds

G. Troy Smith,¹ Eliot A. Brenowitz,^{1,2} Michael D. Beecher,^{1,2} and John C. Wingfield¹

Departments of ¹Zoology and ²Psychology, University of Washington, Seattle, Washington 98195

Seasonal changes in the neural attributes of brain nuclei that control song in songbirds are among the most pronounced examples of naturally occurring plasticity in the adult brain of any vertebrate. The behavioral correlates of this seasonal neural plasticity have not been well characterized, particularly in songbird species that lack adult song learning. To address this question, we investigated the relationship between seasonal changes in gonadal steroids, song nuclei, and song behavior in adult male song sparrows (*Melospiza melodia*). At four times of the year, we measured plasma concentrations of testosterone, neural attributes of song nuclei, and several aspects of song structure in wild song sparrows of a nonmigratory population. We found seasonal changes in the song nuclei that were temporally correlated with changes in testosterone concentrations

and with changes in song stereotypy. Male song sparrows sang songs that were more variable in structure in the fall, when testosterone concentrations were low and song nuclei were small, than in the spring, when testosterone concentrations were higher and song nuclei were larger. Despite seasonal changes in the song nuclei, the song sparrows continued to sing the same number of different song types, indicating that changes in the song nuclei were not correlated with changes in song repertoire size. These results suggest that song stereotypy, but not repertoire size, is a potential behavioral correlate of seasonal plasticity in the avian song control system.

Key words: androgen; seasonal plasticity; bird song; motor stereotypy; song sparrow; song repertoire

Most temperate zone vertebrates breed seasonally. Seasonal changes in brain structures that control reproductive behavior have been reported in numerous vertebrate species (Nottebohm, 1981; Buijs et al., 1986; Boyd and Moore, 1991; Wade and Crews, 1991; Hofman and Swaab, 1992; Skene et al., 1992; Senthilkumaran and Joy, 1993; Lee et al., 1995). Seasonal changes in neural attributes of brain nuclei that control song in songbirds are perhaps the most striking example of naturally occurring plasticity in the adult vertebrate brain (Nottebohm, 1981).

Several attributes of song nuclei change seasonally: (1) overall size (Nottebohm, 1981; Arai et al., 1989; Kirn et al., 1989; Brenowitz et al., 1991; Rucker and Cassone, 1991; Smith et al., 1995); (2) size, density, and number of neurons (Brenowitz et al., 1991; Johnson and Bottjer, 1995; Smith et al., 1995); (3) dendritic and synaptic morphology (DeVoogd et al., 1985; Clower et al., 1989; Hill and DeVoogd, 1991); and (4) incorporation and survival of new neurons (Alvarez-Buylla et al., 1990; Nottebohm et al., 1994). Similar changes in the size of song nuclei have been reported both in captive songbirds in which photoperiod and/or testosterone (T) were manipulated to mimic seasonally changing environmental and hormonal conditions (Nottebohm, 1981; Brenowitz and Arnold, 1985; Arai et al., 1989) and in free-ranging, wild songbirds experiencing natural seasonal changes in environmental cues (Kirn et al., 1989; Brenowitz et al., 1996; Smith, 1996).

Nottebohm (1981) hypothesized that seasonal changes in the song nuclei of canaries (*Serinus canaria*) were related to the seasonal learning and forgetting of songs by adult males. More recent studies, however, have also found seasonal changes in the song nuclei of species that lack adult song learning (Arai et al., 1989; Brenowitz et al., 1991; Smith et al., 1995; Brenowitz et al., 1996; Smith, 1996). These results suggest that although seasonal changes in the song nuclei may be necessary for adult song learning, they are not sufficient.

Several alternative hypotheses for the behavioral correlates of seasonal plasticity in the song control system of species that lack adult song learning have been proposed: (1) song rate, (2) repertoire size, (3) song structure, and (4) song perception (DeVoogd, 1991; Brenowitz and Kroodsma, 1996). These hypotheses are not mutually exclusive.

In the present study, we investigated the hypotheses that changes in song nuclei were related to changes in song repertoire size or song structure. Several studies have suggested that the size of song nuclei is correlated with song repertoire size (Nottebohm et al., 1981; Canady et al., 1984; Kroodsma and Canady, 1985; Brenowitz and Arnold, 1986; DeVoogd et al., 1995), but few studies have investigated whether seasonal changes in the size of song nuclei are associated with changes in repertoire size. Similarly, relatively little is known about seasonal variation in song structure in wild songbirds. In captive male canaries, song is highly stereotyped in structure during the breeding season but becomes more variable in structure in late summer and early fall when song nuclei are smaller (Nottebohm et al., 1986). Few other studies, however, have investigated seasonal changes in the structure of song, particularly in species that lack adult song learning or in wild songbirds under naturalistic conditions.

We examined seasonal patterns of plasma T concentrations, neural attributes of the song nuclei, and song repertoire size and

Received Dec. 12, 1996; revised April 24, 1997; accepted May 22, 1997.

This work was supported by National Institutes of Health Grant MH53032 to E.A.B., National Science Foundation Grants IBN 9408013 and DCB 9005081 to J.C.W., and National Science Foundation Grant IBN 9212175 to M.D.B. G.T.S. was a Howard Hughes Medical Institute Predoctoral Fellow. We thank Roberta Conti and Mark Coleman for assistance with song analysis and Lynn Erckmann for advice and assistance on the testosterone assay. Liz Campbell helped record song behavior, and Karin Lent helped with histology.

Correspondence should be addressed to G. Troy Smith, Department of Zoology, Patterson Laboratories, University of Texas at Austin, Austin, TX 78712.

Copyright © 1997 Society for Neuroscience 0270-6474/97/176001-10\$05.00/0

structure in free-living male song sparrows (*Melospiza melodia morphna*). This species is well suited to study the behavioral correlates of seasonal changes in the song nuclei for two reasons: (1) song sparrows lack adult song learning (Mulligan, 1966; Marler and Peters, 1987) (M. D. Beecher and S. E. Campbell, unpublished observations); and (2) in western Washington State, song sparrows remain on their territories and defend them with song year round, enabling us to study the same population of birds, and to record the songs of the same individual birds, at different times of the year (Wingfield and Hahn, 1994). This study had three goals: (1) to determine whether the size and neural attributes of song nuclei changed seasonally in wild songbirds that sing year round; (2) to identify potential behavioral correlates of changes in the song nuclei in a songbird species that lacks adult song learning; and (3) to determine the relative timing of seasonal changes in plasma concentrations of T, anatomical attributes of song nuclei, and song behavior.

MATERIALS AND METHODS

Experiment 1: plasma testosterone concentrations and neural attributes of song nuclei

Study sites and subjects. We collected territorial adult male song sparrows from three field sites in western Washington State: Skagit State Wildlife Recreation Area, Lee Forest, and Montlake Fill in Seattle. These sites are within 80 km of each other and experience similar weather and photoperiod conditions. We captured males by using a mist net and playback of male song. All collected males entered the net within 15 min of playback onset. We immediately collected blood samples from the alar veins to measure plasma concentrations of T. We stored the blood samples on ice until they were returned to the laboratory, where we centrifuged them, removed the plasma, and stored the plasma at -20°C .

We brought the song sparrows to the University of Washington on the day of capture, deeply anesthetized them with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL), and perfused them with heparinized avian saline followed by 10% neutral buffered formalin (NBF). The brains and testes were removed and stored in 10% NBF. Only males with completely pneumatized skulls, indicating that the birds were adults, were included in the study (Nero, 1951; Serventy et al., 1967).

Sampling dates. We collected male song sparrows at four times of year: (1) in early spring, March 1–31, when the males were preparing to breed and their testes were recrudescing ($n = 6$); (2) in late spring, April–early May, during the peak of the breeding season, when testes were fully recrudescing ($n = 10$); (3) in early fall, September–early October, just after prebasic molt, when the testes were completely regressed, despite high levels of territoriality and song ($n = 7$); and (4) in late fall, December, when spontaneous territorial behavior and spontaneous song were relatively infrequent ($n = 8$) (Wingfield and Hahn, 1994). A similar proportion of males was collected from each field site in each season.

Testosterone assay. We measured plasma concentrations of T by radioimmunoassay (RIA) after extraction and separation from other steroids (see Wingfield and Farner, 1975; Ball and Wingfield, 1987; Smith, 1996). The minimum detectable concentration of T varied between 0.05 and 0.26 ng/ml plasma. Samples containing concentrations below the limit of detection of the RIA were treated as having concentrations at the detection limit for statistical analyses.

Histology and volume measurements. Brains were embedded in gelatin and cryoprotected for 2–3 d in 10% NBF containing 20% sucrose (4°C). The brains were then frozen on dry ice and sectioned at $50\ \mu\text{m}$ on a sliding microtome. Sections were mounted on gelatin-subbed slides, dried overnight, stained in thionin, dehydrated in a graded ethanol series, cleared in xylene, and coverslipped in DPX mountant (BDH Laboratory Supplies, Poole, UK).

Sections were viewed at a final magnification of $46\times$ under a microprojector. In every other $50\ \mu\text{m}$ section, we traced the Nissl-defined borders of five song nuclei: the higher vocal center (HVC), the robust nucleus of the archistriatum (RA), area X of the parolfactory lobe, the lateral portion of the magnocellular nucleus of the anterior neostriatum (IMAN), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). We also traced the borders of the thalamic reticuloid (Rt) and pretectal (Pt) nuclei, which are not involved in song. The Nissl-defined borders of HVC coincide with those defined by other markers of the

nucleus (Johnson and Bottjer, 1993, 1995; Bernard and Ball, 1995; Smith et al., 1997b). We included the caudomedial extension of HVC (para-HVC of Johnson and Bottjer, 1995) in our tracings. Our measures of HVC therefore coincide with the “inclusive” measure of HVC of Kim et al. (1989) and with measures of HVC used in previous studies of European starlings (*Sturnus vulgaris*), white-crowned sparrows (*Zonotrichia leucophrys*), eastern towhees (*Pipilo erythrophthalmus*), and spotted towhees (*Pipilo maculatus*) (Brenowitz et al., 1991; Bernard and Ball, 1995; Smith et al., 1995; Smith, 1996). We used the criteria of DeVoogd et al. (1991) to distinguish the tracheosyringeal from the lingual portion of the hypoglossal nucleus. Tracings of brain regions were digitized with a flat bed scanner, and the areas of tracings were calculated using Image (version 1.57; Wayne Rasband, National Institutes of Health, Bethesda, MD). The volumes of brain nuclei were reconstructed using the formula for the volume of a cone frustum (Krebs et al., 1989; Smith et al., 1995; Smith, 1996).

Neuronal attributes. We measured the cross-sectional area and density of neuronal profiles in HVC and RA by using a systematic random-sampling scheme described previously (Smith et al., 1997a). Approximately 100 neuronal profiles in RA and 125–250 neuronal profiles in HVC were counted and measured in each bird. These sample sizes are sufficient to provide an accurate estimate of mean neuronal size and density in these nuclei (Brenowitz et al., 1995).

As part of a separate study (A. Tramontin, G. T. Smith, C. Breuner, and E. Brenowitz, unpublished observations), we evaluated the accuracy of our neuron-sampling technique. We measured neuronal density in HVC and RA of four late spring and four late fall brains both with the sampling methods of this study and with an optical disector (Gundersen et al., 1988; Coggeshall and Lekan, 1996). There were no significant differences between the neuronal density estimates provided by the sampling method we used and those provided by the unbiased optical disector technique. Our sampling methods therefore yielded accurate estimates of neuron density and number in HVC and RA.

Experiment 2: seasonal changes in song behavior

Study site and subjects. The entire song repertoires of the same five adult males were recorded at each of the times of the year when plasma T concentrations and song nuclei were measured (see above). Songs of color-banded adult male song sparrows were recorded at Discovery Park (Seattle, WA). Playback of male song was used to stimulate the birds to sing in all seasons. Using a Sennheiser MKH 816 T-U directional microphone and a Sony TCD5M cassette recorder, we recorded at least 200 (and usually >300) songs from each song sparrow in each season to ensure that the entire repertoire was sampled (Borror, 1965; Searcy et al., 1985; Podos et al., 1992). We were unable to measure spontaneous song rates from the birds simultaneously, because playback was necessary to stimulate the birds to sing a sufficient number of songs to record their entire repertoires in all seasons.

Terminology. The following terms were used to describe song structure: (1) note, a sound producing a continuous trace on the sound spectrograph; (2) minimal unit of production (MUP), a sequence of notes that always occurred together in the same order in a male's songs (Barlow, 1977; Podos et al., 1992); (3) syllable, an MUP that was serially repeated to form a trill; (4) trill, a rapidly repeated sequence of similar syllables; (5) song type, a distinctive class of songs within the repertoire; songs belonging to the same song type shared many of the same MUPs, especially at the beginning of the song; and (6) song type variation, a rendition of a song type that contains a minor change in the number, presence, or sequence of MUPs.

All song analyses, except frequency–time cross-correlations, were performed on a Kay DSP 5500 sonograph. Spectrograms of each recorded song were viewed, and the sequence of notes in each song was entered into a database.

Repertoire size. Song sparrows sing repertoires of 5–24 distinct song types, although in western Washington, they typically sing <12 song types (Nice, 1943; Borror, 1965; Mulligan, 1966; Searcy et al., 1985; Beecher et al., 1994). Different song types were readily distinguishable both by ear and by visual inspection of sound spectrograms and have also been shown to be statistically distinguishable (Podos et al., 1992). We used sound spectrograms to count the number of different song types sung by each bird in each season.

Song complexity. We measured the complexity of song sparrow songs by counting the number of different note types (MUPs; see definition above) in each song. The number of note types per song has been used in previous studies as an index of song complexity (Read and Weary,

1992; Lampe and Epsmark, 1994). The average number of MUPs per song was compared for each bird across seasons.

Length of trills. Trills are characteristic components of song sparrow song that involve the rapid repetition of a syllable (Mulligan, 1966). For each trill type, we counted the number of syllables in each rendition. Only trill types that were produced in all four seasons were included. The number of syllables per trill was averaged over all trill types for each bird and compared across seasons.

Variability of song types. Although different song types are easily distinguishable from one another, song sparrows do not always produce their song types identically from one rendition to the next. Instead, they produce variations of their song types (Borror, 1965; Mulligan, 1966).

All of the song types produced by an individual song sparrow can usually be recorded by sampling 200–300 songs. Unlike song types, the number of which is finite, the possible number of song type variations produced by an individual song sparrow is practically infinite. Individual male song sparrows continued to produce different variations of their song types even after 1000 songs (Podos et al., 1992).

To quantify the variability with which song sparrows sang their song types, we counted the number of different variations of song types produced in a fixed number of song renditions (6–39, depending on the number of renditions of each song type recorded). For any one song type, the same number of song renditions was analyzed in all four seasons. The rate at which new variations of song types were produced in each season was calculated by dividing the number of different variations in the analyzed songs by the number of songs analyzed. This yielded a value from 0 to 1, in which lower values indicated more stereotypy in song type production (i.e., fewer variations produced in a fixed number of song renditions), and higher values indicated more variability in song type production. A similar analysis has been used in a previous study to quantify song variability (Ball and Nowicki, 1990).

As a second measure of song variability, we determined the proportion of notes shared between different variations within song types by using a modification of methods of Podos et al. (1992). Within each song type in each season, all possible pairs of song variations were compared with each other using the Jaccard correlation coefficient ($CC_{a,b}$) (Sneath and Sokal, 1973; Baulieu, 1989; Podos et al., 1992): $CC_{a,b} = c/(c + u_a + u_b)$, where a and b are the song type variations being compared, c is the number of MUPs common to both variations a and b , u_a is the number of MUPs unique to variation a , and u_b is the number of MUPs unique to variation b . The resulting similarity score was a value from 0 to 1, which indicated the proportion of note types shared by the two song variants. Unlike the study of Podos et al. (1992), we did not truncate songs of unequal lengths before comparing them, because such truncation tends to underestimate the variability within song types. The average pairwise similarity scores were calculated for each song type in each season and then pooled across song types to yield a single average within-song type similarity score for each bird in each season.

Variability of syllable structure. For each bird, we randomly selected one note of each of the following six note types: (1) buzz, a rapid, long, repetitive frequency modulation, producing a continuous trace on the spectrogram; (2) trill, a repeated series of syllables, produced rapidly but not so much as to produce a continuous trace on the spectrogram; (3) introductory note, the first note of a song; (4) frequency-modulated (FM) note, a note with a rapid change in frequency; (5) whistle, a pure tone with little or no FM; and (6) FM-whistle, a note with both FM and pure tone components. The structural similarity of each selected note was analyzed in all four seasons.

To quantify seasonal changes in the stereotypy of the note structure, we used a pairwise frequency–time cross-correlation method, similar to that used by Clark et al. (1987). Ten renditions of each note were randomly selected from recordings in each season. The note renditions were digitized into the SIGNAL sound analysis program (Beeman, 1989), and a frequency–time cross-correlation was performed between each possible pair of the 10 note renditions in each season. The cross-correlations were performed over the frequency range from 100 Hz below the lowest frequency in any rendition of the note in any of the four seasons to 100 Hz above the highest frequency in any rendition of the note in any season. The average pairwise cross-correlation was used as an index of the stereotypy of the notes in each season. High cross-correlations (e.g., ~ 1) indicated that the structures of the 10 note renditions were similar to one another (i.e., stereotyped). Low cross-correlations (e.g., ~ 0) indicated that the 10 note renditions were less similar (i.e., variable). The average pairwise note cross-correlations were

Figure 1. Seasonal changes in plasma T concentrations. Columns represent mean $\pm$ SEM (error bars) plasma T concentrations in male song sparrows collected at each of the four sampling times. Season significantly affected plasma T concentrations (ANOVA, $p < 0.001$). *a–c*, Significant differences between seasons (PLSD, $p < 0.05$). Sample sizes are as follows: early spring, 6; late spring, 9; early fall, 7; late fall, 8.

pooled across all note types to yield a single note cross-correlation value for each bird in each season.

Statistical analyses. Plasma T concentrations did not meet the assumption of homogeneity of variance for an ANOVA. They were therefore log-transformed before being subjected to a one-factor ANOVA, with season as the independent factor and log-transformed plasma T concentrations as the dependent variable. The volumes and neural attributes of brain nuclei were compared across seasons with one-factor ANOVAs, with season as the independent factor and the neural attributes as the dependent factors. Measurements of song behavior were compared between seasons with repeated measures ANOVAs, with the behavioral measurement in each of the four seasons as the repeated, dependent variable. *Post hoc* comparisons between pairs of seasons for all variables were made with Fisher's protected least significant difference (PLSD) tests. An α level of 0.05 was used to judge significance for all tests.

RESULTS

Plasma T concentrations

Plasma T concentrations changed seasonally (Fig. 1; $F_{(3,26)} = 20$; $p < 0.001$). Plasma T concentrations in both early and late fall males were near or below the detection limit of the hormone assay. In early spring males, mean plasma T concentrations were significantly higher than in both fall samples (PLSD, $p < 0.01$). In late spring males, plasma T concentrations were significantly higher than either in the early or late fall (PLSD, $p < 0.001$) or the early spring (PLSD, $p < 0.01$).

Neural attributes of song nuclei

Size of song nuclei

The size of HVC and RA changed seasonally (Fig. 2; $F_{(3,26)} = 7.7$ and 8.5, respectively; $p < 0.001$ for both). HVC was significantly larger in both the early and late spring than in the early or late fall (PLSD, $p < 0.01$ for all spring vs fall comparisons). RA was larger in the late spring than in either the early or late fall (PLSD, $p < 0.01$). In the early spring, the size of RA was intermediate to that of the late spring and the fall. The mean volume of RA in the early spring did not differ significantly from that of either the late spring (PLSD, $p = 0.12$) or the early fall (PLSD, $p = 0.08$) but was significantly greater than that in the late fall (PLSD, $p = 0.04$).

The size of three other song nuclei—area X, nXII, and IMAN—did not change seasonally (Table 1) ($F_{(3,27)} = 1.31$; $p = 0.29$; $F_{(3,20)} = 0.86$; $p = 0.48$; and $F_{(3,24)} = 2.03$; $p = 0.14$,

Figure 2. Seasonal changes in the size of HVC and RA. Columns represent mean $\pm$ SEM (error bars) volume of HVC and RA. Season significantly affected the volume of HVC and RA (ANOVA, $p < 0.001$ for both). *a, b*, Significant differences between seasons (PLSD, $p < 0.05$). Sample sizes are as follows in both graphs: early spring, 5; late spring, 10; early fall, 7; late fall, 8.

respectively). In addition, the size of two brain nuclei not involved in song behavior, Rt and Pt, did not change seasonally (Table 1) ($F_{(3,27)} = 0.04$ and 1.61 ; $p = 0.99$ and 0.21 , respectively). Thus, the seasonal changes in the size of HVC and RA were specific to those nuclei and do not reflect seasonal changes in the size of brain nuclei in general.

Neuronal attributes of RA and HVC

The size and number, but not the density, of HVC neurons changed seasonally (Fig. 3). HVC neurons were significantly larger in the early and late spring than in the early or late fall ($F_{(3,25)} = 7.0$; $p = 0.001$; PLSD, $p < 0.05$ for all spring vs fall comparisons). The number of neurons within the Nissl-defined boundaries of HVC was also significantly greater in the early and late spring than in the early or late fall ($F_{(3,25)} = 7.1$; $p = 0.001$; PLSD, $p < 0.05$ for all spring vs fall comparisons). Neuronal density in HVC did not change seasonally ($F_{(3,25)} = 0.19$; $p = 0.90$).

The size and density, but not the number, of neurons in RA changed seasonally (Fig. 4). RA neurons were significantly larger in the early and late spring than in the early or late fall ($F_{(3,26)} = 13.0$; $p < 0.001$; PLSD, $p < 0.001$ for all spring vs fall comparisons). The density of RA neurons was significantly lower in late

spring than in early or late fall ($F_{(3,26)} = 6.0$; $p = 0.003$; PLSD, $p < 0.01$ for both comparisons); that is, RA neurons were more closely spaced in the fall than in the late spring. The density of RA neurons in the early spring was intermediate to that of the late spring and fall and did not differ significantly from that of the late spring, early fall, or late fall (PLSD, $p = 0.22$, 0.11 , and 0.07 , respectively). The number of neurons in RA did not change seasonally ($F_{(3,26)} = 0.06$; $p = 0.98$).

Song behavior

Repertoire size

The size of the male song sparrows' repertoires did not change seasonally (Table 2) ($F_{(3,12)} = 0.23$; $p = 0.87$). The males sang the same number of different song types in the fall as they did in the spring.

Song complexity

The complexity of song sparrow songs also did not change seasonally. There was no significant effect of season on the number of different note types (MUPs) in each song (Table 2) ($F_{(3,12)} = 0.48$; $p = 0.70$).

Length of trills

The number of syllables in trills changed seasonally (Table 2) ($F_{(3,12)} = 7.03$; $p = 0.006$). *Post hoc* analyses revealed that trills contained significantly more syllables in the early and late spring than in the early or late fall (PLSD, $p < 0.05$ for all spring vs fall comparisons).

Song type stereotypy: rate of song type variations

The rate at which the song sparrows sang new variations of their song types changed seasonally (Table 2) ($F_{(3,12)} = 8.62$; $p = 0.003$). Song sparrows sang fewer variations in a fixed number of song renditions in the late spring than in either the early or late fall (PLSD, $p < 0.01$). Thus, song types were sung more stereotypically from one song rendition to the next in the late spring than in the fall. The rate of production of different song variations in the early spring was intermediate to that of the late spring and fall and did not differ significantly from that in the late spring, early fall, or late fall (PLSD, $p = 0.06$, 0.11 , and 0.07 , respectively).

Song type stereotypy: similarity of song type variations

Although more variations of song types were sung in a fixed number of song renditions in the fall than the late spring, the similarity of those variations to each other did not change seasonally. The proportion of shared notes between different song variations was not significantly affected by season (Table 2) ($F_{(3,12)} = 2.18$; $p = 0.14$).

Stereotypy of note structure

Notes were more stereotyped in structure in the spring than in the fall (Fig. 5). The average pairwise cross-correlation of 10 note renditions in each season was significantly influenced by season (Table 2) ($F_{(3,12)} = 8.19$; $p = 0.003$). Note cross-correlations were significantly higher in early and late spring than in early or late fall (PLSD, $p < 0.05$ for all spring vs fall comparisons). Thus, different renditions of the same note were more similar to each other (i.e., stereotyped) in the spring than in the fall.

DISCUSSION

Seasonal changes in the size and neuronal attributes of the song nuclei in the song sparrows were similar to those observed in

Table 1. Volume of brain nuclei

Brain nucleus	Volume of brain nucleus (mm ³ , mean $\pm$ SEM)			
	Early spring	Late spring	Early fall	Late fall
Area X	2.18 $\pm$ 0.45 (6) ^a	2.40 $\pm$ 0.14 (10)	2.34 $\pm$ 0.27 (7)	1.83 $\pm$ 0.08 (8)
nXIIIs	0.079 $\pm$ 0.005(4)	0.098 $\pm$ 0.010(7)	0.090 $\pm$ 0.011(6)	0.084 $\pm$ 0.006(7)
1MAN	0.183 $\pm$ 0.029(4)	0.135 $\pm$ 0.011(10)	0.137 $\pm$ 0.009(7)	0.142 $\pm$ 0.010(7)
Rt	2.87 $\pm$ 0.14 (6)	2.92 $\pm$ 0.09 (10)	2.92 $\pm$ 0.19 (7)	2.92 $\pm$ 0.11 (8)
Pt	0.115 $\pm$ 0.007(6)	0.131 $\pm$ 0.005(10)	0.142 $\pm$ 0.011(7)	0.134 $\pm$ 0.009(8)

^aNumbers in parentheses indicate sample sizes.

Figure 3. Seasonal changes in HVC neuronal attributes. Columns represent mean $\pm$ SEM (error bars) cross-sectional area of neuronal somata (A), neuronal density (B), or neuronal number (C) in HVC. HVC neuron size (A) and number (C) changed seasonally (ANOVA, $p < 0.01$ for both). Neuronal density in HVC (B) did not change seasonally. *a, b*, Significant differences between seasons (PLSD, $p < 0.05$). Sample sizes are as follows for all panels: early spring, 5; late spring, 10; early fall, 6; late fall, 8.

other species. Both HVC and RA were larger in late spring than in fall male song sparrows. Similar seasonal changes in the size of these brain regions have been observed in several songbird species both in the wild and in captivity (Nottebohm, 1981; Arai et al., 1989; Kirn et al., 1989; Brenowitz et al., 1991; Bernard and Ball, 1995; Smith et al., 1995; Brenowitz et al., 1996; Smith, 1996).

Changes in the size of RA and HVC were accompanied by changes in neuronal attributes. The seasonal increase in the size of RA was mostly attributable to an increase in the size of its

Figure 4. Seasonal changes in RA neuronal attributes. Columns represent mean $\pm$ SEM (error bars) cross-sectional area of neuronal somata (A), neuronal density (B), or neuronal number (C) in RA. RA neuron size (A) and density (B) changed seasonally (ANOVA, $p < 0.01$ for both). The number of neurons in RA (C) did not change seasonally. *a, b*, Significant differences between seasons (PLSD, $p < 0.05$). Sample sizes are as follows for all panels: early spring, 5; late spring, 10; early fall, 7; late fall, 8.

neurons and an increase in the spacing between those neurons. In contrast to RA, the spring growth of HVC was accompanied by increases in the size and number, but not the density, of neurons. These results are similar to those of a study of captive white-crowned sparrows (Smith et al., 1995) and suggest that similar neuronal changes underlie the seasonal changes in the size of these nuclei in both captive and wild songbirds.

Plasma T concentrations changed seasonally in the male song

Table 2. Song attributes

Attribute	Early spring	Late spring	Early fall	Late fall
Repertoire size (number of song types)	7.60 ± 0.75 ^a	7.60 ± 0.75	7.40 ± 0.68	7.60 ± 0.81
Song type complexity (MUPs/song)	7.00 ± 0.56	6.60 ± 0.45	6.80 ± 0.60	6.86 ± 0.77
Trill length (syllables/trill)*	4.45 ± 0.27 ^b	4.62 ± 0.35 ^b	3.81 ± 0.11 ^c	3.88 ± 0.11 ^c
Rate of song type variation (variations/rendition)*	0.61 ± 0.06 ^{b,c}	0.50 ± 0.06 ^b	0.68 ± 0.06 ^c	0.69 ± 0.06 ^c
Variation similarity score (within song type)	0.58 ± 0.03	0.61 ± 0.03	0.56 ± 0.01	0.57 ± 0.03
Syllable cross-correlation*	0.85 ± 0.02 ^b	0.85 ± 0.01 ^b	0.78 ± 0.01 ^c	0.76 ± 0.02 ^c

^aValues are mean ± SEM.^{b,c}Values in same row with different superscripts differ significantly from each other, PLSD, $p < 0.05$.*One-factor repeated measures ANOVA, $p < 0.05$; $n = 5$ for all measures.

sparrows, as reported in a previous study of the same subspecies (Wingfield and Hahn, 1994). That study also found that plasma T concentrations were very low in the fall, began rising in March, and peaked early during the breeding season. Changes in the size of the song nuclei were temporally correlated with these changes in plasma T concentrations. A study of white-crowned sparrows demonstrated that T plays a dominant role in mediating seasonal changes in the size of the song nuclei (Smith et al., 1997a). Consistent with this result, we found that HVC and RA were larger in the late spring, when T concentrations were at their peak, than in the fall, when T concentrations were basal. In the early spring, when T concentrations were still rising, HVC was as large as it was in the late spring. Furthermore, the size and number of neurons in HVC were also at their peak by early spring. This result suggests that in this species HVC may grow rapidly in response to increasing T concentrations in the early spring, and that there may also be a ceiling effect on the actions of T on neural attributes of this nucleus; HVC did not grow further in response to the peak concentrations of T in the late spring. The size of RA in the early spring was intermediate to that of the fall and the late spring, suggesting that RA was growing as T concentrations were rising in the early spring. At this time, RA neurons were already as large as they would be in the late spring, but the density of these neurons was in transition, intermediate to that of the late fall and late spring. These results suggest that the growth of HVC in the spring precedes that of RA.

Previous studies had reported correlations between repertoire size and the size of song nuclei; that is, individuals, populations, and species that sang more different song types tended to have larger song nuclei (Nottebohm et al., 1981; Canady et al., 1984; Kroodsma and Canady, 1985; Brenowitz and Arnold, 1986; DeVogd et al., 1995). We therefore hypothesized that seasonal changes in the size of the song nuclei of male song sparrows might be associated with a seasonal change in repertoire size. The results of the present study, however, do not support this hypothesis; repertoire size remained constant across seasons, despite changes in the size of HVC and RA. Similarly, the complexity of individual songs, as measured by the number of different note types in each song, did not change seasonally. Thus, having larger song nuclei in the spring than in the fall did not enable song sparrows to incorporate more note types into their songs, nor did having smaller song nuclei in the fall lead to a loss of song notes or types.

Three aspects of song structure did change seasonally: trill

length, the rate at which different variations of song types were produced, and the stereotypy of song notes. In the late spring, when T levels were high and HVC and RA were large, trills were longer, and there was less variation both in the production of song types and in the structure of the individual song notes. In the fall, when T levels were basal and the song nuclei were smaller, trills were shorter, and both song types and note structure were more variable. Seasonal changes in trill length have also been noted in white-crowned sparrows (DeWolfe et al., 1974).

Seasonal changes in trill length and in the stereotypy of song types and notes may be related to structural changes in the neural circuitry and musculature that control song. Trills are a particularly demanding component of song for the motor control system to produce, because they involve the rapid repetition of the same syllables and, therefore, of the same motor patterns. This rapid and repetitive contraction of the same muscle groups may be more likely to fatigue both the syringeal muscles and the associated motor and premotor neurons. If this is true, it may be that the smaller and fewer neurons in the song nuclei in the fall are unable to produce longer trills without fatiguing.

Seasonal changes in song type or note stereotypy may be related to changes in neural attributes of the song nuclei. Calvin (1983) suggested that larger assemblages of neurons may enable more precise temporal motor control by increasing the redundancy, and thereby reducing the error, in motor signals. Increases in the number and/or size of neurons in the song nuclei in the spring may similarly enable songbirds to control the syringeal musculature more precisely and thus may allow them to produce more uniform notes and songs from one rendition to the next.

It is also possible that seasonal changes in trill length and note stereotypy may result from direct effects of T on the syringeal muscles. Syringeal muscles contain receptors for T or its metabolites, and T influences both the weight of the syrinx and the activities of acetylcholinesterase and choline acetyltransferase in syringeal muscle (Lieberberg and Nottebohm, 1979; Luine et al., 1980; Harding et al., 1983). Thus, the low T levels in the fall song sparrows may result in relatively atrophied syringeal muscles that are less able to produce stereotyped notes consistently from one rendition to the next or to produce longer trills without fatiguing.

An alternative explanation of the seasonal change in the rate at which different variations were produced is that this results from changes in the rate at which the birds sang. Song sparrows sing at a higher rate in the spring than in the late fall and winter (Nice, 1943; Nowicki and Ball, 1989; Wingfield and Hahn, 1994). As the

Figure 5. Seasonal changes in structural stereotypy of notes. Sound spectrograms of a single note. *Left*, Three renditions of the same pure tone note sung by the same male song sparrow in the late spring. The three renditions contain little or no frequency modulation and are similar to each other in structure. *Right*, Three renditions of the same note as on the *left* sung by the same song sparrow in the early fall. The three early fall renditions contain more frequency modulation and are less similar to each other. Thus, the structure of this note was more stereotyped in the late spring than in the early fall. Calibration, 0.25 sec.

intervals between successive songs increased in the fall, the probability that the birds sang different variations of their songs from one song rendition to the next may have increased. Our data cannot directly address this hypothesis, but we are not aware of any evidence that song stereotypy varies with intersong interval.

We do not yet know the nature of the causal relationships between changes in T concentrations, neural attributes of song nuclei, and changes in song structure. Seasonal changes in plasma T concentrations may induce changes in neural attributes of the song nuclei, which then result in changes in song structure. Alternatively, seasonal changes in T may induce changes in song structure by mechanisms that are independent of changes in the song nuclei, or seasonal changes in song structure may be unrelated to changes in T concentrations. Further studies of the

temporal sequence of neural, hormonal and behavioral changes, particularly during the periods of growth of the song nuclei in the early spring and of regression in the summer, may elucidate the causal relationships between these changes.

Two possible behavioral correlates of seasonal changes in the song nuclei were not measured in this study: song perception and song rate. It is possible that seasonal changes in the ability to perceive song, or in the amount of song produced, are also correlated with seasonal changes in the song nuclei. Cynx and Nottebohm (1992) reported that male zebra finches on long day photoperiods learned a song discrimination task more rapidly than males on short day photoperiods. Thus, seasonal photoperiod cues may regulate the ability of songbirds to perceive and discriminate songs. It is difficult, however, to relate this result to our study, because zebra finches do not breed seasonally and have

not been shown to undergo seasonal or hormonally induced changes in the size or neural attributes of their song nuclei (Immelmann, 1970; Arnold, 1980). No studies have yet examined seasonal changes in song perception in seasonally breeding songbirds.

Song rate changes seasonally in many seasonally breeding songbird species, including song sparrows (Nice, 1943; Davis, 1958; Hiett and Catchpole, 1982; La Pointe and Bédard, 1984; Nowicki and Ball, 1989; Rost, 1992; Wingfield and Hahn, 1994). It is possible that seasonal changes in the song nuclei may be associated with changes in the amount of song produced. In the present study, we did not measure spontaneous song rates, because it was necessary to use playback to stimulate the birds to sing to record and measure the birds' entire repertoires. This hypothesis is indirectly supported by the finding that genetically different morphs of white-throated sparrows (*Zonotrichia albicollis*) differ both in singing rate and in the size of some song nuclei (DeVoogd et al., 1995). Area X, and both lateral and medial MAN were significantly larger in white morph males, which sing more frequently, than in tan morph males, which sing less frequently. Interestingly, the volumes of HVC and RA, the song nuclei that differed seasonally in our study, did not differ significantly between white and tan morphs.

Two lines of evidence, however, suggest that there is no simple correlation between singing rate and the size of HVC and RA. Castrated male zebra finches sang at a much lower rate than gonadally intact male zebra finches, and this effect was partly reversed by treatment with testosterone (Arnold, 1975). Castration, however, slightly increased, rather than decreased, the size of HVC and RA in male zebra finches (Arnold, 1980). Although these results are not directly comparable to seasonal changes in the size of song nuclei in wild, seasonally breeding songbirds, they do demonstrate that dramatic hormonally induced changes in song rates can occur without changes in the size of HVC and RA. A second example is provided by studies of captive white-crowned sparrows. Male sparrows exposed to long day photoperiods in captivity experienced levels of T that were elevated relative to those of short day males but lower than those of wild breeding males (Wingfield and Moore, 1987; Smith et al., 1995). These captive long day males sang frequently, whereas captive short day males sang rarely if at all (Baker et al., 1984; Smith et al., 1995). Despite these differences in song rates, two studies reported that the size of HVC and RA did not differ between captive long day and short day males (Baker et al., 1984; Smith et al., 1995). When captive long day males were given T implants to produce levels of T comparable to those of wild breeding males, their HVC and RA were significantly larger than those of captive short day males (Smith et al., 1995). These results demonstrate that changes in singing rate in seasonally breeding songbirds can occur in the absence of changes in the size of HVC and RA. Thus, although changes in the size of the song nuclei may facilitate changes in song rate, these neural changes are not necessary for modulating song rate.

Song stereotypy may change seasonally both in species that learn new songs as adults and in species that lack adult song learning. We found that two measures of song stereotypy, the production rate of song variations and the structural stereotypy of notes, changed seasonally in song sparrows, a species that lacks adult song learning. Song stereotypy also changes seasonally in male canaries, an open-ended song-learning species (Nottebohm et al., 1986). In both of these species, songs are more stereotyped in structure during the breeding season,

when T concentrations are high and the song nuclei are large, than outside the breeding season, when T concentrations are lower and the song nuclei are smaller. Although the stereotypy of canary song was not quantified as it was in the song sparrow songs of the present study, the increase in the variability of canary song in the fall seems to be qualitatively greater than the increase in variability that we found in wild song sparrows in the fall (see Nottebohm et al., 1986, their Fig. 6). It is during the unstable song period in late summer and early fall that male canaries modify their songs. These results suggest that seasonal changes in song stereotypy may be common both to species such as canaries, which learn new songs as adults, and to species such as song sparrows, which lack adult song learning. In open-ended song-learning species, the increase in song plasticity in the fall may facilitate modifications of song from year to year, whereas in age-limited species, the increased plasticity is either insufficient for song modification or is not used to modify song from one breeding season to the next.

In summary, we found seasonal changes in aspects of song structure in wild male song sparrows that were temporally correlated with changes in plasma T concentrations and with changes in neural attributes of HVC and RA. Further investigation is needed to determine the behavioral significance of seasonal changes in song structure and to identify potential causal relationships between seasonal changes in T, neural attributes of song nuclei, and these aspects of song structure. In particular, attention should be focused on seasonal changes in song stereotypy, which may be a behavioral correlate of neural plasticity in the song system that is common to both age-limited and open-ended song learning species.

REFERENCES

- Alvarez-Buylla A, Kirn J, Nottebohm F (1990) Birth of projection neurons in adult avian brain may be related to perceptual or motor learning. *Science* 249:1444–1446.
- Arai O, Taniguchi I, Saito N (1989) Correlation between the size of song control nuclei and plumage color change in orange bishop birds. *Neurosci Lett* 98:144–148.
- Arnold AP (1975) The effects of castration and androgen replacement on song, courtship, and aggression in zebra finches (*Poephila guttata*). *J Exp Zool* 191:309–326.
- Arnold AP (1980) Effects of androgens on volumes of sexually dimorphic brain regions in the zebra finch. *Brain Res* 185:441–444.
- Baker MC, Bottjer SW, Arnold AP (1984) Sexual dimorphism and lack of a seasonal change in vocal control regions of the white-crowned sparrow brain. *Brain Res* 295:85–89.
- Ball GF, Nowicki S (1990) Assessment of song quality in photorefractory and photosensitive song sparrows. *Anim Behav* 40:986–987.
- Ball GF, Wingfield JC (1987) Changes in plasma levels of sex steroids in relation to multiple broodiness and nest site density in male starlings. *Physiol Zool* 60:191–199.
- Barlow GW (1977) Modal action patterns. In: *How animals communicate* (Sebeok TA, ed), pp 98–134. Bloomington, IN: Indiana University.
- Baulieu FB (1989) A classification of presence/absence based dissimilarity coefficients. *J Classif* 6:233–246.
- Beecher MD, Campbell SE, Stoddard PK (1994) Correlation of song learning and territory establishment strategies in the song sparrow. *Proc Natl Acad Sci USA* 91:1450–1454.
- Beeman K (1989) "SIGNAL" users' guide. Belmont, CA: Engineering Design.
- Bernard DJ, Ball GF (1995) Two histological markers reveal a similar photoperiodic difference in the volume of the high vocal center in male European starlings. *J Comp Neurol* 360:726–734.
- Borror DJ (1965) Song variation in Maine song sparrows. *Wilson Bull* 77:5–37.
- Boyd SK, Moore FL (1991) Gonadectomy reduces the concentrations of

- putative receptors for arginine vasotocin in the brain of an amphibian. *Brain Res* 541:193–197.
- Brenowitz EA, Arnold AP (1985) Lack of sexual dimorphism in steroid accumulation in vocal control regions of duetting songbirds. *Brain Res* 344:172–175.
- Brenowitz EA, Arnold AP (1986) Interspecific comparisons of the size of neural song control regions and song complexity in duetting birds: evolutionary implications. *J Neurosci* 6:2875–2879.
- Brenowitz EA, Kroodsma DE (1996) The neuroethology of bird song. In: *Ecology and evolution of acoustic communication in birds* (Kroodsma DE, Miller E, eds), pp 385–404. New York: Cornell University.
- Brenowitz EA, Nalls B, Wingfield JC, Kroodsma DE (1991) Seasonal changes in avian song nuclei without seasonal changes in song repertoire. *J Neurosci* 11:1367–1374.
- Brenowitz EA, Lent K, Kroodsma DE (1995) Brain space for learned song in birds develops independently of song learning. *J Neurosci* 15:6281–6286.
- Brenowitz EA, Baptista LF, Lent KL, Wingfield JC (1996) Seasonal plasticity of the song system in wild nuttall's white-crowned sparrows. *Soc Neurosci Abstr* 22:1401.
- Buijs RM, P'evet P, Masson-P'evet M, Pool CW, deVries GJ, Canguilhem B, Vivien-Roels B (1986) Seasonal variation in vasopressin innervation in the brain of the European hamster (*Cricetus cricetus*). *Brain Res* 371:193–196.
- Calvin WH (1983) A stone's throw and its launch window: timing precision and its implications for language and hominid brains. *J Theor Biol* 104:121–135.
- Canady RA, Kroodsma DE, Nottebohm F (1984) Population differences in complexity of a learned skill are correlated with the brain space involved. *Proc Natl Acad Sci USA* 81:6232–6234.
- Clark CW, Marler P, Beeman K (1987) Quantitative analysis of animal vocal phonology: an application to swamp sparrow song. *Ethology* 76:101–115.
- Clower RP, Nixdorf BE, DeVoogd TJ (1989) Synaptic plasticity in the hypoglossal nucleus of female canaries: structural correlates of season, hemisphere, and testosterone treatment. *Behav Neural Biol* 52:63–77.
- Coggeshall RE, Lekan HA (1996) Methods for determining numbers of cells and synapses: a case for more uniform standards of review. *J Comp Neurol* 364:6–15.
- Cynx J, Nottebohm F (1992) Role of gender, season, and familiarity in discrimination of conspecific song by zebra finches (*Taeniopygia guttata*). *Proc Natl Acad Sci USA* 89:1368–1371.
- Davis J (1958) Singing behavior and the gonad cycle of the rufous-sided towhee. *Condor* 60:308–336.
- DeVoogd TJ (1991) Endocrine modulation of the development and adult function of the avian song system. *Psychoneuroendocrinology* 16:41–66.
- DeVoogd TJ, Houtman AM, Falls JB (1995) White-throated sparrow morphs that differ in song production rate also differ in the anatomy of some song-related brain areas. *J Neurobiol* 28:202–213.
- DeVoogd TJ, Nixdorf B, Nottebohm F (1985) Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. *Brain Res* 329:304–308.
- DeVoogd TJ, Pyskaty DJ, Nottebohm F (1991) Lateral asymmetries and testosterone-induced changes in the gross morphology of the hypoglossal nucleus in adult canaries. *J Comp Neurol* 307:65–76.
- DeWolfe BB, Kaska DD, Peyton LJ (1974) Prominent variations in the songs of Gambel's white-crowned sparrows. *Bird Banding* 45:224–252.
- Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ (1988) The new stereological tools: disector, fractionator, nucleator, and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 96:857–881.
- Harding CF, Sheridan K, Walters MJ (1983) Hormonal specificity and activation of sexual behavior in male zebra finches. *Horm Behav* 17:111–133.
- Hiett JC, Catchpole CK (1982) Song repertoires and seasonal song in the yellowhammer, *Emberiza citrinella*. *Anim Behav* 30:568–574.
- Hill KM, DeVoogd TJ (1991) Altered daylength affects dendritic structure in a song-related brain region in red-winged blackbirds. *Behav Neural Biol* 56:240–250.
- Hofman MA, Swaab DF (1992) Seasonal changes in the suprachiasmatic nucleus of man. *Neurosci Lett* 139:257–260.
- Immelmann K (1970) Australian finches in bush and aviary. Sydney, Australia: Angus and Robertson.
- Johnson F, Bottjer SW (1993) Hormone-induced changes in identified cell populations of the higher vocal center in male canaries. *J Neurobiol* 24:400–418.
- Johnson F, Bottjer SW (1995) Differential estrogen accumulation among populations of projection neurons in the higher vocal center in male canaries. *J Neurobiol* 26:87–108.
- Kirn JR, Clower RP, Kroodsma DE, DeVoogd TJ (1989) Song-related brain regions in the red-winged blackbird are affected by sex and season but not repertoire size. *J Neurobiol* 20:139–163.
- Krebs JR, Sherry DF, Healy SD, Perry VH, Vaccarino AL (1989) Hippocampal specialization of food-storing birds. *Proc Natl Acad Sci USA* 86:1388–1392.
- Kroodsma DE, Canady RA (1985) Differences in repertoire size, singing behavior and associated neuroanatomy among marsh wren populations have a genetic basis. *Auk* 102:439–445.
- La Pointe G, Bédard J (1984) Seasonal and individual variability of behaviour rates in male Savannah sparrows. *Biol Behav* 9:343–356.
- Lampe HM, Epsmark YO (1994) Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. *Anim Behav* 47:869–876.
- Lee W, Watanabe M, Glass JD (1995) Photoperiod affects the expression of neural cell adhesion molecule and polysialic acid in the hypothalamus of the Siberian hamster. *Brain Res* 690:64–72.
- Lieberberg I, Nottebohm F (1979) High-affinity androgen binding proteins in the syringeal tissues of songbirds. *Gen Comp Endocrinol* 37:286–293.
- Luine V, Nottebohm F, Harding C, McEwen BS (1980) Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. *Brain Res* 192:89–107.
- Marler P, Peters S (1987) A sensitive period for song acquisition in the song sparrow, *Melospiza melodia*: a case of age-limited learning. *Ethology* 76:89–100.
- Mulligan JA (1966) Singing behavior and its development in the song sparrow, *Melospiza melodia*. *Univ Calif Publ Zool* 81:1–76.
- Nero RW (1951) Pattern and rate of cranial "ossification" in the house sparrow. *Wilson Bull* 63:84–88.
- Nice MM (1943) Studies in the life history of the song sparrow II. The behavior of the song sparrow and other passerines. *Trans Linn Soc NY* 6:1–328.
- Nottebohm F (1981) A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science* 214:1368–1370.
- Nottebohm F, Kasparian S, Pandazis C (1981) Brain space for a learned task. *Brain Res* 213:99–109.
- Nottebohm F, Nottebohm ME, Crane L (1986) Developmental and seasonal changes in canary song and their relation to changes in the anatomy of song control nuclei. *Behav Neural Biol* 46:445–471.
- Nottebohm F, O'Loughlin B, Gould K, Yohay K, Alvarez-Buylla A (1994) The life span of new neurons in a song control nucleus of the adult canary brain depends on time of year when these cells are born. *Proc Natl Acad Sci USA* 91:7849–7853.
- Nowicki S, Ball GF (1989) Testosterone induction of song in photosensitive and photorefractory male sparrows. *Horm Behav* 23:514–525.
- Podos J, Peters S, Rudnicki T, Marler P, Nowicki S (1992) The organization of song repertoires in song sparrows: themes and variations. *Ethology* 90:89–106.
- Read AF, Weary DM (1992) The evolution of bird song: comparative analyses. *Philos Trans R Soc Lond [Biol]* 338:165–187.
- Rost R (1992) Hormones and behavior: a comparison of studies on seasonal changes in song production and testosterone plasma levels in the willow tit *Parus montanus*. *Ornis Fenn* 69:1–6.
- Rucker MG, Cassone VM (1991) Song control nuclei of the house sparrow (*Passer domesticus*) are photoperiodic and photorefractory. *Soc Neurosci Abstr* 17:1051.
- Searcy WA, McArthur PD, Yasukawa K (1985) Song repertoire size and male quality in song sparrows. *Condor* 87:222–228.
- Senthilkumar B, Joy KP (1993) Annual variations in hypothalamic serotonin and monoamine oxidase in the catfish *Heteropneustes fossilis* with a note on brain regional differences of day-night variations in gonadal preparatory phase. *Gen Comp Endocrinol* 90:372–382.
- Serventy DL, Nicholls CA, Farner DS (1967) Pneumatization of the cranium of the zebra finch *Taeniopygia castanotis*. *Ibis* 109:570–578.
- Skene DJ, Masson-Pévet M, Pévet P (1992) Seasonal changes in melatonin binding sites in the pars tuberalis of male European hamsters

- and the effect of testosterone manipulation. *Endocrinology* 132:1682–1686.
- Smith GT (1996) Seasonal plasticity in the song nuclei of wild rufous-sided towhees. *Brain Res* 734:79–85.
- Smith GT, Brenowitz EA, Wingfield JC, Baptista LF (1995) Seasonal changes in song nuclei and song behavior in Gambel's white-crowned sparrows. *J Neurobiol* 28:114–125.
- Smith GT, Brenowitz EA, Wingfield JC (1997a) Roles of photoperiod and testosterone in seasonal plasticity of the avian song control system. *J Neurobiol* 32:426–442.
- Smith GT, Brenowitz EA, Wingfield JC (1997b) Seasonal changes in the size of the avian song control nucleus HVC defined by multiple histological markers. *J Comp Neurol* 381:253–261.
- Sneath PHA, Sokal RR (1973) *Numerical taxonomy*. San Francisco: Freeman.
- Wade J, Crews D (1991) The relationship between reproductive state and "sexually dimorphic" brain areas in sexually reproducing and parthenogenetic whiptail lizards. *J Comp Neurol* 309:507–514.
- Wingfield JC, Farner DS (1975) The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. *Steroids* 26:311–327.
- Wingfield JC, Hahn TP (1994) Testosterone and territorial behaviour in sedentary and migratory sparrows. *Anim Behav* 47:77–89.
- Wingfield JC, Moore MC (1987) Hormonal, social and environmental factors in the reproductive biology of free-living male birds. In: *Psychobiology of reproductive behavior: an evolutionary perspective* (Crews D, ed), pp 149–175. Englewood Cliffs, NJ: Prentice-Hall.