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Seasonal and individual variation in singing behavior correlates with alpha 2-noradrenergic receptor density in brain regions implicated in song, sexual, and social behavior

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Abstract

In seasonally breeding male songbirds, both the function of song and the stimuli that elicit singing behavior change seasonally. The catecholamine norepinephrine (NE) modulates attention and arousal across behavioral states, yet the role of NE in seasonally-appropriate vocal communication has not been well-studied. The present study explored the possibility that seasonal changes in alpha 2-noradrenergic receptors (α_2 -R) within song control regions and brain regions implicated in sexual arousal and social behavior contribute to seasonal changes in song behavior in male European starlings (*Sturnus vulgaris*). We quantified singing behavior in aviary housed males under spring breeding season conditions and fall conditions. α_2 -R were identified with the selective ligand [³H]RX821002 using autoradiographic methods. The densities of α_2 -R in song control regions (HVC and the robust nucleus of the arcopallium [RA]) and the lateral septum (LS) were lower in Spring Condition males. α_2 -R densities in the caudal portion of the medial preoptic nucleus (POM) related negatively to singing behavior. Testosterone concentrations were highest in Spring Condition males and correlated with α_2 -R in LS and POM. Results link persistent seasonal alterations in the structure or function of male song to seasonal changes in NE α_2 -Rs in HVC, RA, and LS. Individual differences in α_2 -R in the POM may in part explain individual differences in song production irrespective of the context in which a male is singing, perhaps through NE modification of male sexual arousal.

Keywords

communication; seasonality; norepinephrine; song control system; social behavior; motivation

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1. Introduction

To communicate effectively animals must attend to specific stimuli within the environment and respond with social signals that are appropriate within a particular context and reflective of the animal's internal or endocrine state. In seasonally breeding songbirds that sing throughout the year, the function of song changes seasonally (Marler and Slabbekoorn, 2004; Catchpole and Slater, 2008). During the breeding season (e.g., spring) circulating concentrations of sex steroids are elevated (Wingfield and Farner, 1993; Wingfield, 2006). At this time males are aroused by, direct attention towards, and sing at high rates to attract females or to repel competing males (Catchpole and Slater, 2008). In contrast, outside of the breeding season (e.g., in fall and winter), sex steroid concentrations are basal. At this time, in some species males continue to sing at high rates, but they do not clearly direct song towards or adjust song in response to other individuals. Song in this context likely plays a role in song learning and may function to keep flocks together (e.g., (Feare, 1984; Eens, 1997). Studies of songbirds have provided crucial insights into specific brain regions involved in the learning, production, and auditory processing of vocal behavior (for recent reviews see (Ball et al., 2008; Brainard, 2008; Brenowitz, 2008; Gentner, 2008; Nordeen and Nordeen, 2008; Theunissen et al., 2008; Wild, 2008); however, little is known about how the brain regulates vocal communication so that it occurs within an appropriate seasonal context.

Across vertebrate species, the catecholamine norepinephrine (NE) plays a role in modulating optimal behavioral responses, sensory responsiveness, attention, and arousal across behavioral states (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005; Castelino and Schmidt, 2009). In songbirds, nuclei of the song control system contain NE receptors and synthesizing enzymes (Mello et al., 1998; Ritters and Ball, 2002a; Ritters et al., 2002), and NE modifies neuronal firing and/or immediate early gene expression in several song control nuclei (Cardin and Schmidt, 2004; Castelino and Ball, 2005; Solis and Perkel, 2006; Sizemore and Perkel, 2008). Furthermore, the boundaries of several nuclei (including HVC [used as a proper name] and the robust nucleus of the arcopallium [RA]) are well-defined by the presence of α_2 -Rs (Bernard and Ball, 1995; Ritters and Ball, 2002a; Ritters et al., 2002).

Past work in songbirds implicates NE in male courtship singing in response to females. For example, in male zebra finches NE levels within some song control nuclei correlated positively with courtship singing (Barclay et al., 1992), and NE depletion using the neurotoxin DSP-4 decreased the numbers of song bouts performed and increased the latency for males to initiate song in response to a female (Barclay et al., 1996). In starlings, α_2 -noradrenergic receptor (α_2 -R) densities in song control regions (HVC and RA) were lower in males trapped in spring (when testosterone was high and males sing to attract females or repel males) compared to males trapped in fall (when testosterone was low and song functions to maintain flocks) (Ritters et al., 2002). Together these data highlight a role for NE in song production and show that shifts in NE receptor densities in song control regions accompany seasonal shifts in the function of song.

In male songbirds, data suggest that several NE rich regions outside of the song control system (Mello et al., 1998; Ritters and Ball, 2002b), including the medial preoptic nucleus (POM) and lateral septum (LS), are differentially involved in season-specific singing behavior (Goodson et al., 1999; Heimovics and Ritters, 2005; 2006; 2007; Heimovics et al., 2009). Together these data suggest that in addition to its direct action on the song system, NE may also influence singing behavior through its action on brain areas implicated in sexual motivation and social behavior.

Short term changes in behavioral state (such as sleep versus wakefulness) are regulated by rapid alterations in NE release (Berridge and Waterhouse, 2003; Castelino and Schmidt, 2009). Perhaps longer-term changes, such as persistent seasonal alterations in male sexual arousal and the production of seasonally-appropriate song may in part be explained by seasonal changes in NE receptors. The present study used receptor autoradiography to provide insight into this possibility by examining song production and α_2 -R densities in male European starlings experiencing Spring-like and Fall-like Conditions.

2. Experimental procedures

2.1 Starling capture and housing

Thirty experimental male and ten stimulus female European starlings were captured using fly-in traps between December 2005 and February 2006 on a farm near Madison, Wisconsin. Birds were then housed in single sex groups indoors in stainless steel cages ($91 \times 47 \times 47$ cm) in the University of Wisconsin – Madison Department of Zoology animal facilities. Birds were placed on a photoperiod of 18 hours light (L): 6 hours dark (D) for six weeks, followed by 6 weeks on a photoperiod of 6L:18D. Male starlings exposed to these shifts in photoperiod become “photosensitive”, a condition observed prior to the onset of the breeding season in which males respond to increases in day length with gonadal recrudescence and increased T production (Dawson et al., 2001). All protocols were approved by the University of Wisconsin Institutional Animal Care and Use Committee and adhered to methods approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Hormone treatment

In a previous field study, α_2 -R density in the song system differed in males that were captured at a time of year at which they were photosensitive with low T (outside the breeding season) as compared to photostimulated with high T (within the breeding season (Riters et al., 2002)). In the present study, T and photoperiod manipulations were used to simulate these conditions. To simulate the breeding season, 20 photosensitive males (now referred to as the “Spring Condition” group) received 2 subcutaneous Silastic T-containing implants (14 mm length, i.d. 1.47 mm; o.d. 1.96 mm [Dow Corning, Midland, MI, USA], packed for 10 mm with crystalline T propionate and sealed with Silastic glue [Sigma Aldrich, St. Louis, MO, USA]) and were shifted to 11L:13D (a photoperiod which induces gonadal growth characteristic of the early breeding season (Dawson et al., 2001)). To simulate the non-breeding season, 10 photosensitive males (now referred to as the “Fall Condition” group) received 2 empty Silastic implants and remained on 6L:18L. During implant surgery, birds were anesthetized using isoflurane gas anesthesia and a small incision was made in the skin over the breast muscle. Implants were placed under the skin and the wound was sutured. After recovering on a heated pad, birds were placed back into single-sex cages.

2.3 Behavioral observations

One to two days following implant surgery two flocks of 10 Spring Condition males and one flock of 10 Fall Condition males were placed into separate indoor behavioral observation aviaries. Each aviary contained five nest boxes and branches for perching. Food and water were available *ad libitum*. Males were individually color banded for identification. In Spring Condition some males will occupy nest boxes whereas others will not. Although not the focus of this study, we were initially interested in examining α_2 -R densities in both males with and without nest boxes; therefore, we examined two flocks of Spring Condition males to ensure our sample included adequate numbers of males with and without nest boxes.

Behavioral observations began after a 4-week habituation period and took place between 09:00 and 12:00h.

Flocks were observed on five consecutive days for 30 min on each day. During this time, a point sampling technique was used to determine the proportion of time any member of the flock spent singing (i.e. during each 30-min observation period it was noted at 60-s intervals whether and from where any member of the flock was singing). For purposes that are not the focus of the present paper, both Spring and Fall Condition males were also presented with a female for 30 min each day (an estradiol implanted female for Spring Condition males and a blank (empty) implanted female for Fall Condition males). The order of 30-min observations (with versus without a female present) was counterbalanced across days, and a novel female conspecific was released into the aviary each day.

2.4 Tissue processing

One day after completion of behavioral observations, males were rapidly decapitated and brains collected. For every male, approximately 2mL of trunk blood were collected and centrifuged at 3000G for 30min at 4°C. Blood plasma (supernatant) was then collected and stored at -20°C until assayed for T. Brains were removed, frozen immediately on powdered dry ice, and stored at -80 °C until sectioning. Brains were sectioned using a cryostat. Sections sixteen microns thick were thaw mounted onto gel-coated microscope slides. Six series of slides were collected so that, on each slide, consecutive sections were 80 microns apart. One series was used for α_2 -R autoradiography, and one series was Nissl stained to aid in the identification of brain regions in which α_2 -R were measured in the study reported here. Other series were used for a previously published dopamine D1-like and D2-like receptor autoradiography (Heimovics et al., 2009). The slides were dried and stored at -20 °C until use.

2.5 Autoradiography

α_2 -R were identified by using the highly selective α_2 -R antagonist, [3H]RX821002, which has been described as a radioligand of choice to detect the total population of α_2 -R in several tissues and species, including birds (O'Rourke et al., 1994; Halme et al., 1995; Ruuskanen et al., 2005; Diez-Alarcia et al., 2006; Cornil and Ball, 2008). After drying at room temperature, slides were pre-incubated in buffer (50 nM Tris-HCl, pH 7.5 at 25°C with 1mM MgCl₂) for 30 min at room temperature. Slides were then incubated for one hour at room temperature in 5nM [3H]RX821002 (specific activity: 49.0 Ci/mmol; Amersham Biosciences, Piscataway, NJ, USA) buffer. In parallel, a few slides were incubated in 5nM [3H]RX821002 to which was added phentolamine-HCl 10 μ M (obtained from Sigma-Aldrich, Inc.) to determine the non-specific binding of this ligand. One hour later, the slides were washed twice for five min in ice-cold buffer followed by a quick dip in ice-cold distilled water. Sections were then fan dried, placed in X-ray cassettes and exposed to tritium-sensitive BioMax[®] MR films (Kodak) along with standards (ART-123; American Radiolabeled Chemicals Inc., St Louis, MO) containing concentrations of tritium ranging from 0.00 to 489.1 μ Ci/g. Sections were run in two batches and tissue from birds in different groups was arbitrarily distributed across batches and film cassettes. The films were developed after 12 weeks.

2.6 Quantification

Each of the developed films was scanned (eight-bit, 600 dpi) using an Epson Perfection 1240 U bed scanner connected to a PC computer. Digitized autoradiograms were analyzed using MetaVue software (Fryer Company, Inc., Huntley, IL, USA) following standard procedures (e.g. (Wang et al., 1997; Ritters et al., 2002; Chen and Lawrence, 2003)). Specifically, the tritium standards were used to calibrate the intensity of the scanned images

in terms of radiochemical concentrations in fmol/mg using the following equation: $(X \text{ microCi}/1000 \text{ mg}) \cdot (10^6 \text{ fmol}/Y \text{ mg})$, where X equals the numerical values associated with the standard and Y equals the specific activity of the ligand (49 Ci/mmol). The gray level value of each standard was then measured in MetaVue and the known concentration of radioactivity (in fmol/mg) was assigned to each corresponding gray level. Values for standards spanning the range of optical density of labeled tissue were selected, and the relationship between the gray values and measures of radioactivity was fit to a 3rd degree polynomial equation, which was used to interpolate gray values between points on the standards. Using these calibrated gray levels, the average intensity of specific binding for α_2 -R was determined for two song control regions, HVC and RA, and two regions implicated in social behavior, the POM and LS. In order to more effectively demonstrate any regional specificity of group differences in ligand binding, we additionally measured α_2 -R density in two control regions for which we had no a priori reasons to predict seasonal differences. The first was area X, a song control region in which α_2 -Rs were not found to change seasonally in our past work (Riters et al., 2002) and the bed nucleus of the stria terminalis, a region involved in social behavior (e.g., (Goodson, 2005)) for which we found no literature that would lead us to hypothesize that α_2 -Rs would change seasonally. Locations of each nucleus were identified based on (Heimovics and Riters, 2007); Figure. 1) and verified using landmarks visible on both autoradiograms and/or adjacent Nissl-stained sections.

Typically, specific binding is determined by subtracting non-specific binding values from total binding values. However in this experiment (as in past studies examining on α_2 -Rs in songbirds [e.g., (Riters et al., 2002)]), no non-specific labeling was observed in any region in sections treated with phentolamine, indicating that the labeling observed was specific to α_2 -R. Within each region, total binding was quantified by measuring the average intensity of calibrated gray levels within a circular region located within the boundaries of each nucleus (visible in adjacent Nissl stained tissue) on 3 serial sections bilaterally. In cases of tissue damage or uneven exposure of autoradiograms, average intensity was measured on a fourth section. If tissue damage and/or uneven exposure was extensive, that individual was dropped from quantification for that brain area. For all individuals, specific binding values were averaged separately for each region and the mean used for statistical analysis.

Additional quantification was performed for both HVC and POM based on past work showing HVC α_2 -R binding densities to differ along the rostro-caudal extent (Riters et al., 2002) and data indicating distinct functions for rostral and caudal portions of POM in male sexual behavior (Balthazart et al., 1998; Riters et al., 2004; Taziaux et al., 2006; Balthazart and Ball, 2007). Specifically, α_2 -R binding densities were quantified within 3 subdivisions of HVC and 4 subdivisions of POM. For HVC, bilateral measurements were made in the most rostral, intermediate, and caudal sections of HVC. Measures were taken from 3 sequential sections within each subdivision of HVC. For POM, bilateral measurements were made in sections in the most rostral portion of POM (just ventromedial to the tractus septopallioesencephalicus [TSM]), in rostral-intermediate POM (just posterior to the level of the full extension of TSM), in caudal-intermediate POM (just prior to the appearance of the anterior commissure [AC]) and in the caudal POM (ventral to the AC). Measures were taken from 3 serial sections within each subdivision of POM. Analyses were performed on the mean of each subdivision of HVC and POM and the mean of the measurements taken for the entire nucleus.

2.7 Testosterone Assay

Plasma testosterone was measured using a commercially available enzyme immunoassay kit (Cayman Chemical 582701) used to assay T in multiple songbird species (e.g., (Pryke et al., 2007; Gil et al., 2008; Sughrue et al., 2008; McGuire and Bentley, 2010; Kelm et al., in

press)). Samples were run in duplicate with respect to a standard curve (limit of detection: 80% B/B₀: 6 pg/ml; sensitivity: 50% B/B₀: 32 pg/ml). The within assay coefficient of variation was 6.87%. A set of identical internal standards was run in each assay. The antibody used in this assay is highly specific for testosterone but has some cross-reactivity with other androgens (5- α DHT: 27.4%; A4:3.7%).

2.8 Statistical treatment of the data

Paired comparisons, t-tests and where appropriate Mann-Whitney U tests, indicated that α_2 -R densities did not differ significantly in any brain region in males with and without nest boxes, thus for all analyses these animals were combined to form the Spring Condition. The mean proportion of min at which each male was singing (described in methods above) on each of the 5 test days was calculated. Means were then arcsine transformed as appropriate for proportion data (Lehner, 1996). Multiple regression analyses were then performed to examine the extent to which male condition (Fall or Spring) and song production contributed statistically to variation in α_2 -R densities in the focal regions HVC, RA, LS and POM and additionally the two control regions area X and the bed nucleus of the stria terminalis.

A separate set of analyses were run to examine the possibly functionally distinct subdivisions of HVC and POM (as suggested by (Balthazart et al., 1998; Ritters et al., 2002; Ritters et al., 2004; Taziaux et al., 2006)). Repeated measures ANOVAs were used to compare α_2 -R densities across subdivisions of HVC and POM, with significant differences followed by Fisher LSD post hoc tests. Multiple regression analyses were also performed to examine the contribution of male condition and song production to α_2 -R densities in subdivisions of HVC and POM.

Testosterone data for Fall Condition males were not normally distributed therefore comparisons between Spring and Fall Condition males were made using non-parametric Mann-Whitney U tests. Spearman correlations were also used to examine relationships between testosterone and α_2 -R densities for birds in Spring and Fall Conditions combined. For the testosterone analyses, data were dropped for 4 individuals for which samples were contaminated with red blood cells (1 Fall Condition, 3 Spring Condition).

For all analyses differences in sample sizes reflect missing data due to tissue damage. For multiple regression analyses the results of both forward and backward stepwise analyses were identical for all brain regions except in the analysis of LS. For LS, forward and backward analyses yielded the same significant effects and we report the results of forward regression based on the higher R² and lower standard error.

3. Results

3.1 Contribution of condition and song production to α_2 -R densities

Results of a multiple regression analysis revealed a significant contribution of male condition (Spring versus Fall) but not song production to mean HVC α_2 -R density (regression results: n = 26, adj R² = 0.18, p = 0.019, significant contribution of male condition: beta = 0.46, beta se = 0.18) with denser HVC α_2 -R observed in Fall compared to Spring Condition males (Figure 2 and 3). Similar results were obtained for α_2 -R density in RA (regression results: n = 27, adj R² = 0.22, p = 0.008, significant contribution of male condition: beta = 0.50, beta se = 0.18; Figure 2 and 3) and in LS (regression results: n = 25, adj R² = 0.23, p = 0.009, significant contribution of male condition: beta = 0.51, beta se = 0.18; Figure 2 and 3). No significant contributing variables were identified in α_2 -R densities for mean POM, area X, or the bed nucleus of the stria terminalis.

3.2 Mean α_2 -R densities in subdivisions of HVC and POM

A repeated measures ANOVA indicated that α_2 -R density increases in a rostral to caudal direction in HVC (mean density [sd]: rostral HVC, 86.07 [14.17]; intermediate HVC, 101.62 [23.40]; caudal HVC, 133.04 [33.36]; $F_{2,42} = 62.11$, $p < 0.001$, with Fisher LSD post hoc tests revealing significant differences between each subdivision). When Breeding Condition was included as a factor, significant differences in α_2 -R densities were observed across subdivisions ($F_{2,40} = 55.60$, $p < 0.001$). Both Breeding Condition ($F_{1,20} = 4.03$, $p = 0.058$) and the subdivision by breeding condition interaction ($F_{2,40} = 2.58$, $p = 0.088$) approached but did not reach statistical significance.

A repeated measures ANOVA indicated that α_2 -R density is highest within intermediate portions of POM (mean density [sd]: rostral POM, 242.57 [48.63]; rostral-intermediate POM, 305.80 [62.07]; caudal-intermediate POM, 293.46 [66.56]; caudal POM, 242.42 [51.52]; $F_{3,75} = 50.68$, $p < 0.001$, with Fisher LSD post hoc tests revealing significant differences between both of the intermediate subdivisions and rostral POM as well as caudal POM). When Breeding Condition was included as a factor, significant differences in α_2 -R densities were observed across subdivisions ($F_{3,72} = 42.25$, $p < 0.001$); however, neither Breeding Condition nor the interaction were significant ($p > 0.62$ in each case).

3.3 Contribution of condition and song production to α_2 -R densities in HVC and POM subdivisions

Results of multiple regression analyses revealed a significant contribution of male condition (Spring versus Fall) but not song production to α_2 -R densities in both intermediate and caudal portions of HVC (intermediate HVC regression results: $n = 26$, $\text{adj } R^2 = 0.20$, $p = 0.012$, significant contribution of male condition: $\beta = 0.48$, $\beta \text{ se} = 0.18$; caudal HVC regression results: $n = 24$, $\text{adj } R^2 = 0.15$, $p = 0.033$, significant contribution of male condition: $\beta = 0.44$, $\beta \text{ se} = 0.19$) with denser HVC α_2 -R observed in Fall compared to Spring Condition males. No significant contributors were identified for α_2 -R density in rostral HVC.

For both intermediate and caudal POM, song production but not male condition statistically contributed to variation in α_2 -R density (intermediate POM regression results: $n = 30$, $\text{adj } R^2 = 0.11$, $p = 0.044$, significant contribution of song production: $\beta = -0.37$, $\beta \text{ se} = 0.18$; caudal POM regression results: $n = 30$, $\text{adj } R^2 = 0.17$, $p = 0.013$, significant contribution of song production: $\beta = -0.45$, $\beta \text{ se} = 0.17$; Figure 4). No significant contributors were identified for α_2 -R density in rostral or rostral intermediate POM.

3.4 Testosterone (T)

A Mann-Whitney U test comparing Fall Condition (blank implanted; $n = 9$) and Spring Condition (T implanted; $n = 17$) males verified the effects of T implants. T was significantly higher in Spring compared to Fall Condition males (MWU = 5.0, $z = 3.85$, $p = 0.0001$; Spring mean = 1539.96 pg/mL, $\text{sd} = 711.68$; Fall mean = 153.86 pg/mL, $\text{sd} = 436.15$ [2 of 9 males had detectable T]). Correlation analyses revealed a significant negative correlation between T and α_2 -R densities in LS ($r = -0.56$, $p = 0.009$; Figure 5). A positive relationship was found between T and α_2 -R densities for the mean of all subdivisions of POM ($r = .53$, $p = 0.006$; Figure 5). The results were similar for each of the subdivisions of POM (rostral POM, $r = 0.51$, $p = 0.014$; rostral-intermediate POM, $r = 0.47$, $p = 0.028$; caudal-intermediate POM, $r = 0.43$, $p = 0.033$; caudal POM $r = 0.52$, $p = 0.008$). These correlations were influenced by the Fall Condition males who had low to undetectable T values. When only birds in Spring Condition were examined no significant correlations were found between T and α_2 -R densities. No other significant correlations were identified for any of

the brain regions examined when all males were considered together or divided according to condition.

4. Discussion

The results of the present study link alterations in α_2 -R densities in regions within and outside of the song control system to seasonally appropriate or individual differences in male song production. Determining directional and causal relationships between seasonal changes in α_2 -R densities and song production awaits future direct manipulations; however the present results provide a strong step toward understanding seasonal changes in α_2 -Rs and ways in which these changes may contribute to changes in singing behavior.

4.1 Functional role of condition-dependent variation in α_2 -R densities

α_2 -R densities were lower in Spring Condition compared to Fall Condition males in song control regions HVC and RA and a region implicated in sexual and social behavior, LS. The findings for HVC and RA are consistent with past studies of male starlings experiencing natural photoperiods and T fluctuations in the wild, with elevated T and a longer photoperiod associated with low density α_2 -R in both HVC and RA (Riters et al., 2002). HVC and RA are important for the arrangement of temporal units of song (Margoliash, 1997). In male starlings structural aspects of song, including bout length and repertoire size have been found to increase in spring (Riters et al., 2000; Van Hout et al., 2009), and in other songbird species measures of song stereotypy and numbers of learned syllables change seasonally in association with season and changes in the function of song (e.g., (Nottebohm, 1981; Nottebohm et al., 1981; Smith et al., 1997a)). Based on previous studies, NE stimulation of α_2 -R in male zebra finches reduced spontaneous activity in RA (Solis and Perkel, 2006) and reduced input to RA from LMAN (Sizemore and Perkel, 2008), which is a region implicated in context-specific adult song variability (Kao and Brainard, 2006; Kao et al., 2008). α_2 -Rs are found both pre- and post-synaptically, with the pre-synaptic receptors functioning as auto-receptors, inhibiting NE release (Starke, 2001). Thus, down-regulation of α_2 -R within HVC and RA of Spring Condition males may function to enhance overall NE activity to directly (or perhaps indirectly by influencing another neurochemical systems (Sizemore and Perkel, 2008)) stimulate structurally appropriate features of song used to attract a female or repel a male.

Although not a “song control” nucleus, data suggest LS context-dependently modifies male song production. Lesions to LS can stimulate or inhibit courtship song depending upon whether the species examined is territorial or gregarious (Goodson et al., 1999), and activity in LS as indicated by immediate early gene markers differs in male starlings singing in Spring and Fall Conditions (Heimovics and Riters, 2006; 2007). The present data suggest alterations in α_2 -Rs in LS may play a role in altering LS regulation of song seasonally. Furthermore, α_2 -Rs in LS inhibit male sexual arousal in rats (Gulia et al., 2002), thus downregulation of α_2 -R within LS of Spring Condition males may function to facilitate seasonally appropriate sexual responses to females, including sexually-motivated song in spring.

4.2 α_2 -R densities in POM relate to song production but not seasonal condition

In contrast to the patterns observed for HVC, RA, and LS, our data indicate that song production rather than male condition best predicted statistical variation in α_2 -R densities in both intermediate and caudal portions of POM. Several studies implicate the POM in sexually-motivated male song production in Spring Condition starlings (Riters and Ball, 1999; Heimovics and Riters, 2005; Alger and Riters, 2006; Alger et al., 2009). A growing number of studies also indicate that the role of the POM extends to song in Fall Condition

males (Riters et al., 2005; Alger and Riters, 2006; Heimovics et al., 2009) and song used to defend nesting territories from other males in Spring Condition birds (Heimovics et al., 2009). Past work in male zebra finches shows that NE depletion using the neurotoxin DSP-4 substantially reduced NE in the medial preoptic area (as well as other areas) and resulted in a delay in the initiation of singing behavior (Barclay et al., 1996). These findings were interpreted as reflecting a role for NE in arousal or attention needed to initiate song production. The POM is critically involved in male sexual arousal across vertebrates (Crews, 2005; Balthazart and Ball, 2007; Hull and Dominguez, 2007; Ball and Balthazart, 2010), and studies in quail suggest that NE in the POM inhibits male sexual behavior (Bailhache et al., 1993). It is thus possible that increasing α_2 -R densities (which may result in a reduction in NE activity, through pre-synaptic α_2 -auto-receptor inhibition (Starke, 2001)) in caudal POM may underlie seasonally appropriate increases in sexual behavior, including sexually-motivated song. Indeed although seasonal condition did not contribute significantly to statistical variation in α_2 -R densities, Spring Condition males generally had higher densities of α_2 -R in caudal POM than Fall Condition males (Figure 4). Thus by modifying an individual's state of sexual arousal, individual differences in α_2 -R densities in POM may underlie individual differences in song production.

4.3 The role of testosterone in condition-dependent differences in α_2 -R densities

As expected, T was significantly elevated in Spring (T implanted) compared to Fall (blank implanted) Condition males. Thus the seasonal changes in α_2 -R in HVC, RA, and LS may be modified directly by seasonal fluctuations in T. In support of this idea, in the present study T concentrations correlated with α_2 -R densities in LS and POM; however these correlations were not observed when males in Fall or Spring Condition (the condition in which T varied most) were considered alone. Correlations between T and α_2 -R densities were not found for HVC or RA; however, it is possible that T induced alterations in α_2 -R are not linear for these regions, but that once T crosses a certain threshold α_2 -R densities drop. Links between T and noradrenergic systems have been documented in several past studies in birds (Harding et al., 1983; Barclay and Harding, 1988; Balthazart and Ball, 1989; Ball and Balthazart, 1990; Barclay and Harding, 1990). HVC, RA, LS, and caudal POM all contain receptors for androgens or androgen metabolites (Gahr, 2001). The extent to which T plays a direct role in altering α_2 -R densities in these regions must be determined in future research.

T also plays an important role in mediating seasonal changes in neural plasticity of the song control system (Ball et al., 2004; Brenowitz, 2004; Meitzen and Thompson, 2008). T treatment increases the volumes of HVC and RA (Tramontin et al., 2003), whereas castration rapidly reduces the volumes of these regions (Thompson et al., 2007). Given the seasonal expansion and contraction of HVC and RA it may be that as the volume of a nucleus increases this causes a stable population of receptors to spread out resulting in decreased density; however, past work in male starlings does not support this possibility. Specifically, increases in the volumes of HVC and RA were not accompanied by proportionally equivalent decreases in α_2 -R density (Riters et al., 2002). Furthermore, changes in volume and density do not occur synchronously, rather increases in volume begin earlier in spring than decreases in α_2 -R density (Riters et al., 2002). Although not extensively studied, the volume of LS has not been found to change seasonally in non-food storing birds (Shiflett et al., 2002). Thus differences found between Fall and Spring Condition males in α_2 -R densities in HVC, RA, as well as LS likely reflect seasonal changes in receptor numbers or binding affinity, but this must be determined in future work.

4.4 The role of photoperiod in condition-dependent alterations in α_2 -R densities

Similar to birds living in the wild, the birds in Spring and Fall Condition in the present study differed not only in testosterone concentrations but also with respect to the photoperiods to

which they were exposed (11L:13D for Spring Condition males and 6L:18D for Fall Condition males). Past studies in songbirds indicate that small seasonal changes in the volumes of HVC and RA can occur independently from T concentrations (Smith et al., 1997b; Dloniak and Deviche, 2001; Tramontin et al., 2001; Riters et al., 2002; Caro et al., 2005). Thus, in the present study, as well as in the wild, differences in the photoperiods to which Spring and Fall Condition males were exposed may have contributed to differences in receptor density.

4.5 α_2 -R densities vary regionally within HVC and POM

Similar to past results, α_2 -Rs were denser in rostral than caudal HVC (Riters et al., 2002). Furthermore, differences in α_2 -density in Spring and Fall Condition males were restricted to intermediate and caudal portions of HVC. This suggests that HVC may possess functionally distinct rostral and caudal subdivisions and that seasonal alterations in α_2 -R specifically in intermediate and caudal HVC are particularly important for seasonal adjustments of song structure.

α_2 -R densities also differed along the rostrocaudal axis in POM, with the densest receptor concentrations restricted to intermediate rather than rostral or caudal POM. These findings are consistent with previous studies highlighting distinct subdivisions within POM. For example, both results of lesion studies and studies using immediate early genes suggest that the rostral POM is more critical for the regulation of sexual interest and that the caudal portion is critical for copulation (Balthazart et al., 1998; Riters and Ball, 1999; Riters et al., 2004; Taziaux et al., 2006). The present data showing that α_2 -R in intermediate and caudal POM but not rostral or rostral-intermediate POM contributed to statistical variance in male song production offer further support to prior work identifying distinct functional topography within the POM (reviewed in (Balthazart and Ball, 2007)).

4.6 α_2 -R densities did not relate to condition or song in all regions

α_2 -R densities in area X and the bed nucleus of the stria terminalis did not correlate with song behavior, testosterone or seasonal condition. These regions were not expected to display seasonal changes in α_2 -R (i.e., past studies do not suggest seasonal changes in α_2 -Rs in these areas). They were included as a control to assess the extent to which group differences in ligand binding were regionally specific. The failure to find changes as a function of condition for these regions indicates that differences observed in HVC, RA, and LS as well as correlations between song and POM are confined to select regions for which we had a priori reasons to predict a role for NE in song or social behavior (as reviewed in the introduction).

4.7 Conclusions

The links between α_2 -R and brain regions involved in song control and social behavior are consistent with the idea that persistent seasonal alterations in the structure or function of male song may in part be explained by seasonal changes in NE α_2 -Rs in HVC, RA, and LS. The results of this study also identify for the first time a context-independent linear relationship between song and α_2 -R in the POM, suggesting that individual differences in a male's propensity to sing within any context may be regulated by α_2 -R activity within this region, perhaps through NE modification of male sexual arousal. This finding more broadly contributes to the understanding of the mechanisms underlying consistent individual variation in behavior (or "personality"), a poorly understood topic that is receiving increasing attention (e.g., (Ball and Balthazart, 2008; Williams, 2008; Duckworth, 2010)). Altogether these findings are consistent with the hypothesis that seasonal alterations and individual differences in α_2 -R densities in regions within and outside of the song control system may modulate NE activity to support seasonally appropriate or individual differences

in singing behavior; however, the precise causal relationships among these variables and specific contributions of NE activity within each region to behavior await future research.

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References

- Alger SJ, Maasch SN, Ritters LV. Lesions to the medial preoptic nucleus affect immediate early gene immunolabeling in brain regions involved in song control and social behavior in male European starlings. *European Journal of Neuroscience*. 2009; 29:970–982. [PubMed: 19245371]
- Alger SJ, Ritters LV. Lesions to the medial preoptic nucleus differentially affect singing and nest box-directed behaviors within and outside of the breeding season in European starlings (*Sturnus vulgaris*). *Behav Neurosci*. 2006; 120:1326–1336. [PubMed: 17201478]
- Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci*. 2005; 28:403–450. [PubMed: 16022602]
- Bailhache T, Surlemont C, Balthazart J. Effects of neurochemical lesions of the preoptic area on male sexual behavior in the Japanese quail. *Brain Res Bull*. 1993; 32:273–283. [PubMed: 8374807]
- Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Ritters LV, Balthazart J. Seasonal plasticity in the song control system: multiple brain sites of steroid hormone action and the importance of variation in song behavior. *Ann N Y Acad Sci*. 2004; 1016:586–610. [PubMed: 15313796]
- Ball GF, Balthazart J. Steroid Modulation of Muscarinic Cholinergic and alpha2-Adrenergic Receptor Density in the Nucleus Intercollicularis of the Japanese Quail. *Eur J Neurosci*. 1990; 2:828–835. [PubMed: 12106090]
- Ball GF, Balthazart J. Individual variation and the endocrine regulation of behaviour and physiology in birds: a cellular/molecular perspective. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:1699–1710. [PubMed: 18048288]
- Ball GF, Balthazart J. 2010Sexual arousal, is it for mammals only? *Horm Behav*.
- Ball, GF.; Ritters, LV.; MacDougall-Shackleton, SA.; Balthazart, J. Sex differences in brain and behavior and the neuroendocrine control of the motivation to sing. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 320-331.
- Balthazart J, Absil P, Gerard M, Appeltants D, Ball GF. Appetitive and consummatory male sexual behavior in Japanese quail are differentially regulated by subregions of the preoptic medial nucleus. *J Neurosci*. 1998; 18:6512–6527. [PubMed: 9698339]
- Balthazart J, Ball GF. Effects of the noradrenergic neurotoxin DSP-4 on luteinizing hormone levels, catecholamine concentrations, alpha 2-adrenergic receptor binding, and aromatase activity in the brain of the Japanese quail. *Brain Res*. 1989; 492:163–175. [PubMed: 2568873]
- Balthazart J, Ball GF. Topography in the preoptic region: differential regulation of appetitive and consummatory male sexual behaviors. *Front Neuroendocrinol*. 2007; 28:161–178. [PubMed: 17624413]
- Barclay SR, Harding CF. Androstenedione modulation of monoamine levels and turnover in hypothalamic and vocal control nuclei in the male zebra finch: steroid effects on brain monoamines. *Brain Res*. 1988; 459:333–343. [PubMed: 2460189]
- Barclay SR, Harding CF. Differential modulation of monoamine levels and turnover rates by estrogen and/or androgen in hypothalamic and vocal control nuclei of male zebra finches. *Brain Res*. 1990; 523:251–262. [PubMed: 1698105]
- Barclay SR, Harding CF, Waterman SA. Correlations between catecholamine levels and sexual behavior in male zebra finches. *Pharmacol Biochem Behav*. 1992; 41:195–201. [PubMed: 1371609]

- Barclay SR, Harding CF, Waterman SA. Central DSP-4 treatment decreases norepinephrine levels and courtship behavior in male zebra finches. *Pharmacol Biochem Behav.* 1996; 53:213–220. [PubMed: 8848453]
- Bernard DJ, Ball GF. Two histological markers reveal a similar photoperiodic difference in the volume of the high vocal center in male European starlings. *J Comp Neurol.* 1995; 360:726–734. [PubMed: 8801262]
- Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev.* 2003; 42:33–84. [PubMed: 12668290]
- Brainard, M. The anterior forebrain pathway and vocal plasticity. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 240-255.
- Brenowitz EA. Plasticity of the adult avian song control system. *Ann N Y Acad Sci.* 2004; 1016:560–585. [PubMed: 15313795]
- Brenowitz, EA. Plasticity of the song control system in adult birds. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 332-349.
- Cardin JA, Schmidt MF. Noradrenergic inputs mediate state dependence of auditory responses in the avian song system. *J Neurosci.* 2004; 24:7745–7753. [PubMed: 15342742]
- Caro SP, Lambrechts MM, Balthazart J. Early seasonal development of brain song control nuclei in male blue tits. *Neurosci Lett.* 2005; 386:139–144. [PubMed: 16051436]
- Castelino CB, Ball GF. A role for norepinephrine in the regulation of context-dependent ZENK expression in male zebra finches (*Taeniopygia guttata*). *Eur J Neurosci.* 2005; 21:1962–1972. [PubMed: 15869489]
- Castelino CB, Schmidt MF. What birdsong can teach us about the central noradrenergic system. *J Chem Neuroanat.* 2009
- Catchpole, CK.; Slater, PJB. *Bird song: biological themes and variations*. Cambridge, [England]: Cambridge University Press; 2008.
- Chen F, Lawrence AJ. The effects of antidepressant treatment on serotonergic and dopaminergic systems in Fawn-Hooded rats: a quantitative autoradiography study. *Brain Res.* 2003; 976:22–29. [PubMed: 12763618]
- Cornil CA, Ball GF. Interplay among catecholamine systems: dopamine binds to alpha2-adrenergic receptors in birds and mammals. *J Comp Neurol.* 2008; 511:610–627. [PubMed: 18924139]
- Crews D. Evolution of neuroendocrine mechanisms that regulate sexual behavior. *Trends Endocrinol Metab.* 2005; 16:354–361. [PubMed: 16139506]
- Dawson A, King VM, Bentley GE, Ball GF. Photoperiodic control of seasonality in birds. *J Biol Rhythms.* 2001; 16:365–380. [PubMed: 11506381]
- Diez-Alarcia R, Pilar-Cuellar F, Paniagua MA, Meana JJ, Fernandez-Lopez A. Pharmacological characterization and autoradiographic distribution of alpha2-adrenoceptor antagonist [3H]RX 821002 binding sites in the chicken brain. *Neuroscience.* 2006; 141:357–369. [PubMed: 16697114]
- Dloniak SM, Deviche P. Effects of testosterone and photoperiodic condition on song production and vocal control region volumes in adult male dark-eyed juncos (*Junco hyemalis*). *Horm Behav.* 2001; 39:95–105. [PubMed: 11243737]
- Duckworth RA. Evolution of personality: Developmental constraints on behavioral flexibility. *Auk.* 2010; 127:752–758.
- Eens M. Understanding the complex song of the European starling: An integrated approach. *Adv Study Beh.* 1997; 26:355–434.
- Feare, CJ. *The Starling*. Oxford: Oxford Press; 1984.
- Gahr M. Distribution of sex steroid hormone receptors in the avian brain: functional implications for neural sex differences and sexual behaviors. *Microsc Res Tech.* 2001; 55:1–11. [PubMed: 11596145]
- Gentner, TQ. Temporal auditory pattern recognition in songbirds. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 187-198.

- Gil D, Bulmer E, Celis P, Puerta M. Increased sibling competition does not increase testosterone or corticosterone levels in nestlings of the spotless starling (*Sturnus unicolor*). *Horm Behav.* 2008; 54:238–243. [PubMed: 18190915]
- Goodson JL. The vertebrate social behavior network: evolutionary themes and variations. *Horm Behav.* 2005; 48:11–22. [PubMed: 15885690]
- Goodson JL, Eibach R, Sakata J, Adkins-Regan E. Effect of septal lesions on male song and aggression in the colonial zebra finch (*Taeniopygia guttata*) and the territorial field sparrow (*Spizella pusilla*). *Behav Brain Res.* 1999; 101:167–180. [PubMed: 10342406]
- Gulia KK, Kumar VM, Mallick HN. Role of the lateral septal noradrenergic system in the elaboration of male sexual behavior in rats. *Pharmacol Biochem Behav.* 2002; 72:817–823. [PubMed: 12062571]
- Halme M, Sjoholm B, Savola JM, Scheinin M. Recombinant human alpha 2-adrenoceptor subtypes: comparison of [3H]rauwolscine, [3H]atipamezole and [3H]RX821002 as radioligands. *Biochim Biophys Acta.* 1995; 1266:207–214. [PubMed: 7742388]
- Harding CF, Sheridan K, Walters MJ. Hormonal specificity and activation of sexual behavior in male zebra finches. *Horm Behav.* 1983; 17:111–133. [PubMed: 6862388]
- Heimovics SA, Cornil CA, Ball GF, Ritters LV. D1-like dopamine receptor density in nuclei involved in social behavior correlates with song in a context-dependent fashion in male European starlings. *Neuroscience.* 2009:962–973. [PubMed: 19356680]
- Heimovics SA, Ritters LV. Immediate early gene activity in song control nuclei and brain areas regulating motivation relates positively to singing behavior during, but not outside of, a breeding context. *J Neurobiol.* 2005; 65:207–224. [PubMed: 16155901]
- Heimovics SA, Ritters LV. Breeding-context-dependent relationships between song and cFOS labeling within social behavior brain regions in male European starlings (*Sturnus vulgaris*). *Horm Behav.* 2006; 50:726–735. [PubMed: 16914152]
- Heimovics SA, Ritters LV. ZENK labeling within social behavior brain regions reveals breeding context-dependent patterns of neural activity associated with song in male European starlings (*Sturnus vulgaris*). *Behav Brain Res.* 2007; 176:333–343. [PubMed: 17113163]
- Hull EM, Dominguez JM. Sexual behavior in male rodents. *Horm Behav.* 2007; 52:45–55. [PubMed: 17499249]
- Kao MH, Brainard MS. Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability. *J Neurophysiol.* 2006; 96:1441–1455. [PubMed: 16723412]
- Kao MH, Wright BD, Doupe AJ. Neurons in a forebrain nucleus required for vocal plasticity rapidly switch between precise firing and variable bursting depending on social context. *J Neurosci.* 2008; 28:13232–13247. [PubMed: 19052215]
- Kelm CA, Forbes-Lorman RM, Auger CJ, Ritters LV. Mu-opioid receptor densities are depleted in regions implicated in agonistic and sexual behavior in male European starlings (*Sturnus vulgaris*) defending nest sites and courting females. *Behav Brain Res.* in press.
- Lehner, PN. *Handbook of Ethological Methods*. Cambridge: Cambridge University Press; 1996.
- Margoliash D. Functional organization of forebrain pathways for song production and perception. *J Neurobiol.* 1997; 33:671–693. [PubMed: 9369466]
- Marler, PR.; Slabbekoorn, H. *Nature's Music: The Science of Birdsong*. Academic Press; 2004.
- McGuire NL, Bentley GE. A functional neuropeptide system in vertebrate gonads: Gonadotropin-inhibitory hormone and its receptor in testes of field-caught house sparrow (*Passer domesticus*). *Gen Comp Endocrinol.* 2010; 166:565–572. [PubMed: 20074575]
- Meitzen J, Thompson CK. Seasonal-like growth and regression of the avian song control system: neural and behavioral plasticity in adult male Gambel's white-crowned sparrows. *Gen Comp Endocrinol.* 2008; 157:259–265. [PubMed: 18457836]
- Mello CV, Pinaud R, Ribeiro S. Noradrenergic system of the zebra finch brain: immunocytochemical study of dopamine-beta-hydroxylase. *J Comp Neurol.* 1998; 400:207–228. [PubMed: 9766400]
- Nordeen, EJ.; Nordeen, KW. Circuits and cellular mechanisms of sensory acquisition. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 256-270.

- Nottebohm F. A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science*. 1981; 214:1368–1370. [PubMed: 7313697]
- Nottebohm F, Kasparian S, Pandazis C. Brain space for a learned task. *Brain Res*. 1981; 213:99–109. [PubMed: 7237153]
- O'Rourke MF, Blaxall HS, Iversen LJ, Bylund DB. Characterization of [3H]RX821002 binding to alpha-2 adrenergic receptor subtypes. *J Pharmacol Exp Ther*. 1994; 268:1362–1367. [PubMed: 7908054]
- Pryke SR, Astheimer LB, Buttemer WA, Griffith SC. Frequency-dependent physiological trade-offs between competing colour morphs. *Biol Lett*. 2007; 3:494–497. [PubMed: 17609174]
- Riters LV, Ball GF. Lesions to the medial preoptic area affect singing in the male European starling (*Sturnus vulgaris*). *Horm Behav*. 1999; 36:276–286. [PubMed: 10603291]
- Riters LV, Ball GF. Sex differences in the densities of alpha 2-adrenergic receptors in the song control system, but not the medial preoptic nucleus in zebra finches. *J Chem Neuroanat*. 2002a; 23:269–277. [PubMed: 12048110]
- Riters LV, Ball GF. Sex differences in the densities of alpha(2)-adrenergic receptors in the song control system, but not the medial preoptic nucleus in zebra finches. *J Chem Neuroanat*. 2002b; 23:269–277. [PubMed: 12048110]
- Riters LV, Eens M, Pinxten R, Ball GF. Seasonal changes in the densities of alpha(2) noradrenergic receptors are inversely related to changes in testosterone and the volumes of song control nuclei in male European starlings. *J Comp Neurol*. 2002; 444:63–74. [PubMed: 11835182]
- Riters LV, Eens M, Pinxten R, Duffy DL, Balthazart J, Ball GF. Seasonal changes in courtship song and the medial preoptic area in male European starlings (*Sturnus vulgaris*). *Horm Behav*. 2000; 38:250–261. [PubMed: 11104643]
- Riters LV, Schroeder MB, Auger CJ, Eens M, Pinxten R, Ball GF. Evidence for opioid involvement in the regulation of song production in male European starlings. *Behavioral Neuroscience*. 2005; 119:245–255. [PubMed: 15727529]
- Riters LV, Teague DP, Schroeder MB, Cummings SE. Vocal production in different social contexts relates to variation in immediate early gene immunoreactivity within and outside of the song control system. *Behav Brain Res*. 2004; 155:307–318. [PubMed: 15364491]
- Ruuskanen JO, Laurila J, Xhaard H, Rantanen VV, Vuoriluoto K, Wurster S, Marjamäki A, Vainio M, Johnson MS, Scheinin M. Conserved structural, pharmacological and functional properties among the three human and five zebrafish alpha 2-adrenoceptors. *Br J Pharmacol*. 2005; 144:165–177. [PubMed: 15655522]
- Shiflett MW, Gould KL, Smulders TV, DeVogd TJ. Septum volume and food-storing behavior are related in parids. *J Neurobiol*. 2002; 51:215–222. [PubMed: 11984843]
- Sizemore M, Perkel DJ. Noradrenergic and GABA B receptor activation differentially modulate inputs to the premotor nucleus RA in zebra finches. *J Neurophysiol*. 2008; 100:8–18. [PubMed: 18463188]
- Smith GT, Brenowitz EA, Beecher MD, Wingfield JC. Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. *J Neurosci*. 1997a; 17:6001–6010. [PubMed: 9221796]
- Smith GT, Brenowitz EA, Wingfield JC. Roles of photoperiod and testosterone in seasonal plasticity of the avian song control system. *J Neurobiol*. 1997b; 32:426–442. [PubMed: 9087894]
- Solis MM, Perkel DJ. Noradrenergic modulation of activity in a vocal control nucleus in vitro. *J Neurophysiol*. 2006; 95:2265–2276. [PubMed: 16371453]
- Starke K. Presynaptic autoreceptors in the third decade: focus on alpha2-adrenoceptors. *J Neurochem*. 2001; 78:685–693. [PubMed: 11520889]
- Sughrue KM, Brittingham MC, French JBJ. Endocrine effects of the herbicide linuron on the American goldfinch (*Carduelis tristis*). *The Auk*. 2008; 125:411–419.
- Taziaux M, Cornil CA, Dejace C, Arckens L, Ball GF, Balthazart J. Neuroanatomical specificity in the expression of the immediate early gene c-fos following expression of appetitive and consummatory male sexual behaviour in Japanese quail. *Eur J Neurosci*. 2006; 23:1869–1887. [PubMed: 16623844]

- Theunissen, FE.; Amin, N.; Shaevitz, S.; Woolley, SMN.; Fremouw, T.; Hauber, ME. Song selectivity and the songbird brain. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 157-173.
- Thompson CK, Bentley GE, Brenowitz EA. Rapid seasonal-like regression of the adult avian song control system. *Proc Natl Acad Sci U S A*. 2007; 104:15520–15525. [PubMed: 17875989]
- Tramontin AD, Perfito N, Wingfield JC, Brenowitz EA. Seasonal growth of song control nuclei precedes seasonal reproductive development in wild adult song sparrows. *Gen Comp Endocrinol*. 2001; 122:1–9. [PubMed: 11352547]
- Tramontin AD, Wingfield JC, Brenowitz EA. Androgens and estrogens induce seasonal-like growth of song nuclei in the adult songbird brain. *J Neurobiol*. 2003; 57:130–140. [PubMed: 14556279]
- Van Hout AJ, Eens M, Balthazart J, Pinxten R. Complex modulation of singing behavior by testosterone in an open-ended learner, the European Starling. *Horm Behav*. 2009; 56:564–573. [PubMed: 19800345]
- Wang Z, Young LJ, Liu Y, Insel TR. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. *J Comp Neurol*. 1997; 378:535–546. [PubMed: 9034909]
- Wild, MJ. Birdsong: Anatomical foundations and central mechanisms of sensorimotor integration. In: Ziegler, HP.; Marler, PR., editors. *Neuroscience of Birdsong*. Cambridge: Cambridge University Press; 2008. p. 136-152.
- Williams TD. Individual variation in endocrine systems: moving beyond the ‘tyranny of the Golden Mean’. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:1687–1698. [PubMed: 18048295]
- Wingfield, JC. Communicative behaviors, hormone-behavior interactions, and reproduction in vertebrates. In: Neill, JD., editor. *Knobil and Neill’s Physiology of Reproduction*. St. Louis, San Diego, London: Elsevier; 2006. p. 1995-2030.
- Wingfield, JC.; Farner, DS. Endocrinology of reproduction in wild species. In: Farner, DS., et al., editors. *Avian Biology*. Vol. 9. London: Academic Press; 1993. p. 163-247.

Figure 1.

Illustrations of coronal sections from one hemisphere of the starling brain. Sections A–E progress from rostral to caudal. Circles indicate approximate areas in which α_2 -R densities were quantified. Abbreviations: A, arcopallium; BST, bed nucleus of the stria terminalis; Cb, cerebellum; CO, optic chiasm; CoA, anterior commissure; Gct, mesencephalic central gray; HA, apical part of the hyperpallium; HD, densocellular part of the hyperpallium; MSt, medial striatum; LS, lateral septum; mMAN, medial magnocellular nucleus of the anterior nidopallium; MS, medial septum; NIII, third cranial nerve; N, nidopallium; NC, caudal nidopallium; POM, medial preoptic nucleus; RA, robust nucleus of the arcopallium; Rt, nucleus rotundus; TnA, nucleus taeniae of the amygdala; V, ventricle; VTA, ventral tegmental area.

Figure 2. Representative photomicrographs showing densities of α_2 -R in HVC (top), RA (middle), and LS (bottom) in a Fall Condition (left) and Spring Condition (left) male. Photomicrographs were scanned and imported into Adobe Photoshop Elements 6.0. The contrast for each image was enhanced identically for all individuals being compared.

Figure 3.

Bar graphs illustrating the mean (+sem) α_2 -R densities in HVC (the mean for all sections quantified), RA, and LS. Open bars represent Fall Condition males. Filled bars represent Spring Condition males. Dots overlying each bar represent the mean α_2 -R density measure for each individual bird. Sample sizes are indicated in each bar. * = $p < 0.05$.

Figure 4.

Scatterplot illustrating significant contribution of singing behavior to α_2 -R densities in caudal POM. Untransformed data are shown to illustrate actual values. Analyses were performed on arcsine transformed difference scores (see text for details). Each circle represents an individual bird. Open circles = Fall Condition males; Filled circles = Spring Condition males.

Figure 5. Scatterplots illustrating significant correlations between testosterone concentrations and α_2 -R densities in LS and POM. Each circle represents an individual bird. Open circles = Fall Condition males; Filled circles = Spring Condition males.