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Soft song during aggressive interactions: Seasonal changes and endocrine correlates in song sparrows

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ABSTRACT

It is well known that songbirds produce high amplitude songs ("broadcast songs"). Songbirds also produce low amplitude songs ("soft songs") during courtship or territorial aggression in the breeding season. Soft songs are important social signals but have been studied far less than broadcast songs. To date, no studies have examined seasonal changes in soft song or its endocrine regulation. Here, in male song sparrows, we examined soft songs during a simulated territorial intrusion in the breeding season and non-breeding season. We also measured plasma testosterone and dehydroepiandrosterone (DHEA) levels in subjects immediately after the aggressive encounter. The total number of songs produced (broadcast + soft songs) did not vary between seasons. However, there was a dramatic increase in the percentage of soft song in the non-breeding season. Further, the percentage of soft song was negatively correlated with plasma testosterone levels in the non-breeding season. There were seasonal differences in the acoustic structure of two major elements of soft song, trills and buzzes. The minimum frequency of trills was lower in the non-breeding season, and the element repetition rate of buzzes was lower in the non-breeding season. To our knowledge, this is the first study to (1) examine soft songs outside of the breeding season and (2) to identify endocrine correlates of soft songs, which are important social signals in songbirds.

Introduction

In songbirds, song is important for both mate attraction and territorial defense (Catchpole and Slater, 2008). Traditionally, researchers have focused on high amplitude songs (hereafter broadcast songs) that transmit over a large distance to potentially numerous receivers. Many songbirds also produce low amplitude songs (hereafter soft songs, but also known as short range songs, quiet songs, whispered songs) that are performed at close range during courtship or territorial disputes (Anderson et al., 2007, 2008, 2012; Dabelsteen, 1990; Dabelsteen and Pedersen, 1988; Dabelsteen et al., 1998; Hof and Hazlett, 2010; Reichard et al., 2011; Titus, 1998). Soft song is a highly aggressive signal in black-throated blue warblers (*Dendroica caerulescens*) (Hof and Hazlett, 2010), swamp sparrows (*Melospiza georgiana*) (Ballentine et al., 2008), and song sparrows (*Melospiza melodia*) (Searcy et al., 2006).

Male song sparrows have a repertoire of 5–16 types of broadcast songs (Borror, 1965; Hiebert et al., 1989; Mulligan, 1966; Podos et al., 1992), and this repertoire of broadcast songs crystallizes during the first year of life (Marler and Peters, 1987). Broadcast songs are typically produced in the range of 80–85 dB SPL. Soft songs, by contrast, are sung at much lower amplitudes, commonly less than 70 dB SPL (range 50–77 dB SPL; Anderson et al., 2008). Soft songs can be broadcast song types that are simply sung at low amplitude (crystallized soft song) or can be distinct from any song type in the broadcast song repertoire (warbled soft song) (Anderson et al., 2008). In general, soft songs can be distinguished from broadcast songs by their low amplitude, by the inclusion of note types that are not common in the broadcast song repertoire, and by their increased minimum frequency, maximum frequency, and frequency bandwidth (Anderson et al., 2008). In song sparrows, broadcast song functions in territory defense (Beecher et al., 1994; Nowicki et al., 1998) and mate attraction (O'Loghlen and Beecher, 1999; Reid et al., 2004), while soft songs appear to be produced exclusively during male–male aggressive encounters and do not appear to function in courtship (Anderson et al., 2007). This suggests that soft songs may be used preferentially in the non-breeding season, when males are not actively attracting female mates.

Although soft songs are important social signals, they have been studied far less than broadcast songs. In particular, no studies have specifically examined the incidence or acoustic structure of soft songs outside of the breeding season, nor the endocrine regulation of soft

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song. In contrast, many studies have quantified the incidence and structure of songs across different seasons and endocrine contexts, typically focusing on broadcast song or without distinguishing between soft song and broadcast song (e.g., Ball and Balthazart, 2010; Ball et al., 2004; Brenowitz, 2004; Meitzen et al., 2007; Smith et al., 1997a,b; Soma et al., 2002, 2004). In song sparrows, song stereotypy (presumably of broadcast songs) decreases in the non-breeding season, coincident with decreases in the sizes of several song control nuclei in the forebrain and plasma testosterone levels (Smith et al., 1997b). These data suggest that soft song might also vary seasonally and be regulated by steroid hormones; in particular, stereotypy or vocal performance might decrease in the non-breeding season.

Here, we investigated seasonal differences in soft song incidence and structure as well as plasma steroid levels in a sedentary population of male song sparrows that defend territories and sing year-round. During a simulated territorial intrusion (STI) that included conspecific song playback and a live caged decoy, we recorded soft songs from free-living subjects, with one cohort in the breeding season and another in the non-breeding season. We analyzed characteristics of whole soft songs, as well as characteristics of trills and buzzes within soft songs.

Trills and buzzes are common acoustic elements in both broadcast and soft songs in the song sparrow, and they are rapidly modulated in the frequency domain (Anderson et al., 2008). Trill production requires precise coordination of the syrinx and respiratory system with the beak movements that modify resonances in the vocal tract (Nowicki et al., 1992; Podos, 1996; Suthers and Goller, 1997). This coordination places a physical limit on a bird's ability to simultaneously maximize beak gape (thereby maximizing the frequency range of repeated syllables) and trill rate (the rate at which repeating syllables are produced; Podos and Nowicki, 2004). By plotting trill rate versus frequency bandwidth, one can estimate a performance limit, with distance from this limit taken as a measure of vocal performance (Ballentine et al., 2004; Podos, 2001). Male songbirds modulate vocal performance in an aggressive context (DuBois et al., 2009) and respond differently to songs differing in vocal performance (Cramer and Price, 2007; DuBois et al., 2011; Illes et al., 2006), suggesting that vocal performance of trills is a salient signal in male–male communication. In addition, we explored whether buzzes show a similar relationship between frequency bandwidth and element repetition rate and whether the vocal performance of trills or buzzes varies seasonally.

We also measured plasma testosterone and dehydroepiandrosterone (DHEA) levels in subjects immediately after the aggressive encounter, because both steroids are important in the control of territorial singing in song sparrows (Newman and Soma, 2011; Pradhan et al., 2010; Soma et al., 2002, 2008).

Materials and methods

Subjects

Subjects were territorial adult male song sparrows near Vancouver, British Columbia. Males were considered territorial if they responded aggressively to conspecific song playback. Two separate groups of subjects were tested in the field during two seasons: (1) breeding season (June 29–July 3, 2009; $n=8$); (2) non-breeding season (Dec. 23–29, 2009; $n=8$). The breeding season lasts from early April to late July, and the non-breeding season lasts from early October to early February. This population is sedentary, and males maintain territories year-round (Soma, 2006; Wingfield and Hahn, 1994). Territorial aggression, including singing behavior, is high during both seasons (Newman and Soma, 2011; Wingfield and Hahn, 1994). Song sparrows are closed-ended learners, and the song repertoire does not vary across the seasons (e.g., Smith et al., 1997b).

Field protocol

Songs were recorded from subjects responding to a 20-min simulated territorial intrusion (STI) as in Wingfield and Hahn (1994). After identification of a territorial male, a speaker was placed face up on the ground immediately beside a caged live decoy, which was covered. A mist net was set up within the territory and then furled and pushed near the ground. We recorded the songs of subjects using a Sennheiser ME67 shotgun microphone and recorded onto a Marantz PMD-661 compact solid-state recorder (Marantz America, Inc., Westbury, NY, USA) (16 bit, 44,100 samples/s sampling rate). The tip of the microphone barrel was placed 1 m from the center of the decoy cage (24 in wide, 17 in high, 14 in deep), with the barrel pointing directly at the top of the cage. Subjects approached the decoy and landed on the decoy cage during STIs, allowing us to obtain high-quality recordings of soft songs.

Conspecific song was played at a volume judged by ear to be within the normal range of broadcast song (approximately 80–85 dB SPL) throughout the 20 min trial. Songs for playbacks were recorded from 4 wild breeding male song sparrows (not subjects) near Vancouver, British Columbia. Each playback consisted of 5–6 distinct song types from a single male song sparrow. Each song type repeated 10 times at 10 s intervals with a 1 min pause between different song types. Playback stimuli were arranged and filtered with Syrinx-PC 2.6 h developed by John Burt (available at http://www.syrinxpc.com/).

During an STI, two observers (CJM, KKS) followed the trial at a distance of approximately 10 m. One observer (CJM) followed the subject with binoculars and quietly signaled when the subject sang. The other observer (KKS) wrote down the time of each song, whether it was a soft song or a broadcast song, and the approximate distance of the subject from the decoy. Songs were categorized as a soft song or broadcast song by ear. Experienced observers in the field can reliably categorize songs as broadcast or soft at a single cutoff of 77 dB SPL (Anderson et al., 2008), and this method has been used in previous studies of soft song (Akçay et al., 2011; Ballentine et al., 2008; Hof and Hazlett, 2010; Searcy et al., 2006). Our categorization of soft song was conservative; in the rare case where the categorization of a song was unclear, the song in question was categorized as a broadcast song.

After the 20 min STI, we continued to use song playback and quickly unfurled the mist net to capture the subject. Subjects that we were able to catch ($n=6$ in the breeding season, $n=8$ in the non-breeding season) were captured within 5.5 min, with an average of 1.1 min to capture (no significant difference between seasons, $p=0.25$). After the subject was captured, we quickly collected blood from the brachial vein (~300 μL) into heparinized capillary tubes. All subjects were bled within 7 min of capture, with an average of 4.2 min to bleed (no significant difference between seasons, $p=0.23$).

Measurement of plasma testosterone and DHEA levels

Steroids were extracted from plasma samples using solid phase extraction with C_{18} columns as described previously (Newman et al., 2008a), with minor modifications (Taves et al., 2010, 2011). Columns were primed with 3 mL HPLC-grade ethanol and equilibrated with 10 mL deionized water. Plasma samples (22.5 μL for testosterone, 33 μL for DHEA) were diluted with 10 mL deionized water and loaded onto the columns. Samples were washed with 10 mL 40% HPLC-grade methanol in water, eluted with 5 mL 90% HPLC-grade methanol in water, and then dried at 40 °C in a vacuum centrifuge (ThermoElectron SPD111V Speedvac). Samples were resuspended in assay buffer with 0.5% absolute ethanol for testosterone assay (Charlier et al., 2009) or 5% absolute ethanol for DHEA assay (Newman et al., 2008a).

Testosterone and DHEA were measured in duplicate using commercial radioimmunoassay kits (testosterone: Beckman Coulter DSL-4100; DHEA: Beckman Coulter DSL-8900). Both of these assays