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Robust behavioral effects of song playback in the absence of testosterone or corticosterone release

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ABSTRACT

Some species of songbirds elevate testosterone in response to territorial intrusions while others do not. The search for a general explanation for this interspecific variation in hormonal response to social challenges has been impeded by methodological differences among studies. We asked whether song playback alone is sufficient to bring about elevation in testosterone or corticosterone in the dark-eyed junco (*Junco hyemalis*), a species that has previously demonstrated significant testosterone elevation in response to a simulated territorial intrusion when song was accompanied by a live decoy. We studied two populations of juncos that differ in length of breeding season (6–8 vs. 14–16 weeks), and conducted playbacks of high amplitude, long-range song. In one population, we also played low amplitude, short-range song, a highly potent elicitor of aggression in juncos and many songbirds. We observed strong aggressive responses to both types of song, but no detectable elevation of plasma testosterone or corticosterone in either population. We also measured rise in corticosterone in response to handling post-playback, and found full capacity to elevate corticosterone but no effect of song class (long-range or short-range) on elevation. Collectively, our data suggest that males can mount an aggressive response to playback without a change in testosterone or corticosterone, despite the ability to alter these hormones during other types of social interactions. We discuss the observed decoupling of circulating hormones and aggression in relation to mechanisms of behavior and the cues that may activate the HPA and HPG axes.

Introduction

Hormones are key mediators of social behavior, in part because they provide a flexible link to allow appropriate responses to changes in the environment, including photoperiod, food, and the presence of conspecifics (Adkins-Regan, 2005). This interaction has bidirectional causality: hormones can facilitate the expression of behavior, and exhibiting a behavior can likewise affect the release of hormones. Among the best-studied hormone-behavior interactions in vertebrates is that between testosterone and aggressive behavior expressed during male-male social challenges in the breeding season. In some species, social challenges activate the hypothalamo–pituitary–gonadal (HPG) axis, leading to the release of testosterone (T) from the gonads and elevating circulating T levels above baseline breeding levels (Archer, 2006; Goymann, 2009; Wingfield et al., 1990). This acute elevation in T is thought to be beneficial during periods of social instability, e.g. by mobilizing energy reserves and shifting stress reactivity, immune function, or behavior (Muehlenbein and Bribiescas, 2005; Wingfield et al., 2001). The hormonal effects of social challenges are best characterized in birds, where, interestingly, a growing number of studies indicate that T does not always elevate in response to social challenges (reviewed in Goymann, 2009). This interspecific variability has been linked with various ecological factors, such as the relative importance of parental care and the length of the breeding season (Goymann, 2009; Landys et al., 2007; Lynn, 2008). In some species that do not elevate T in response to a social challenge, activation of the hypothalamo–pituitary–adrenal (HPA) axis has been observed instead, particularly in species that experience a short breeding season (Landys et al., 2007). These data suggest that socially-induced HPA axis activation may interfere with HPG axis signaling, or that glucocorticoids, such as corticosterone (CORT), mediate physiological and behavioral responses to social challenges in some species. While often thought of as a 'stress hormone', CORT induces a variety of organism-wide metabolic and behavioral effects that would be well suited to social challenges, e.g. increased activity, energy metabolism, or cardiovascular function, and the suppression of sickness behavior (Ashley et al., 2009; Breuner et al., 1998; Haller et al., 2008; Landys et al., 2004; Sapolsky et al., 2000).

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Methodological differences among studies have further complicated interpretations of interspecific variation in the hormonal effects of social challenge (Goymann, 2009). Studies have varied, for example, in the length of time males were exposed to simulated territorial intrusions (STI). While most studies report T or CORT elevation 10 to 30 min after the initiation of an STI (Landys et al., 2007; McGlothlin et al., 2008; Van Duyse et al., 2004; Wingfield and Wada, 1989), other species may require significantly more time to elevate hormone levels (Wikelski et al., 1999). In addition, the type of decoy (taxidermic or live) has varied from study to study and may affect hormone signaling (Scriba and Goymann, 2008, 2010), suggesting that different sensory modalities may influence the degree to which a social challenge leads to an elevation in T or CORT.

A key issue that is not completely resolved is whether song playback alone is sufficient to activate the HPA or HPG axes. Over twenty years ago, two studies suggested that vocal cues may be insufficient to elicit a robust T surge following an STI, though both studies were somewhat limited in interpretive power due to small sample sizes. Wingfield and Wada (1989) reported significant T elevation in free-living song sparrows (*Melospiza melodia*) in response to a live bird supplemented with song playback, whereas the presentation of either a devocalized male or song playback alone only trended toward T elevation. Captive male cowbirds (*Molothrus ater*) housed with either devocalized or normally vocalizing males had higher T than males exposed to silence or auditory playback alone (Dufty and Wingfield, 1990). A few recent studies report no relationship between the duration of playback exposure and the degree of T or CORT elevation (Busch et al., 2008; Deviche et al., 2006; Fokidis et al., 2011) or no effect of song playback on T (Deviche et al., 2012). Because of the high interspecific variation in the hormonal effects of social challenges (Goymann, 2009), however, failure to find an effect of playback alone on T or CORT can be difficult to interpret unless these null results are contrasted with data demonstrating social elevation of T does occur under other conditions. Thus, our study investigates the issue of whether song alone can lead to T or CORT elevation in a species in which past work has shown a rise in T under other circumstances (see below).

To this end, we tested the hormonal effects of playback in two subspecies of dark-eyed junco (*Junco hyemalis*). The junco is a North American sparrow that has long been a model system for the study of life history, behavior and hormones, particularly the role of T in mediating life history and phenotypic evolution (Ketterson et al., 1992, 2009; Reed et al., 2006). We focused on the well-studied slate-colored junco (*J. h. carolinensis*) breeding in the Appalachian Mountains in Virginia (VA) and the comparatively less well-studied pink-sided junco (*J. h. mearnsi*) that breeds around the Yellowstone plateau and Teton range in Wyoming (WY). During the breeding season, we challenged free-living males with song playbacks previously recorded in their native population, and compared their post-challenge circulating T and CORT levels to controls that were captured rapidly. In one subspecies (WY), we also asked whether restraint stress further elevated CORT to test whether individuals exposed to song playback had reached their maximum CORT levels. Finally, we asked whether hormone elevation (or lack thereof) differed in response to high amplitude, long-range song versus low amplitude, short-range song that has been shown to elicit an extremely aggressive behavioral response in these and other Emberizid sparrows (Beecher et al., 2000; Reichard et al., 2011).

The primary goal of our study was to determine whether T or CORT rises in response to a social challenge consisting solely of a song playback in the absence of a decoy. While not identical to our study, a previous study in the same VA population revealed elevated T in response to a live conspecific and song playback in male juncos captured during the female's fertile period (McGlothlin et al., 2008), suggesting that male juncos can elevate T in response to some social challenges. By measuring the hormonal effect of song playback in two junco populations, we further examined whether geographic differences in latitude and length of breeding season could affect the elevation of T or CORT in response to playback (Landys et al., 2007; Wingfield et al., 2007). Finally, by presenting males with two song stimuli that differ significantly in the strength of the behavioral response they elicit (Reichard et al., 2011), we asked how different types of social challenges may differentially affect the HPA and HPG axes.

Materials and methods

Study sites and populations

We conducted two song playback experiments with free-living, male dark-eyed juncos. The experiments took place in the area surrounding Mountain Lake Biological Station in Pembroke, Virginia (VA; 37°22′N, 80°32′W), from 24 April to 25 May 2011 and in Grand Teton National Park, Wyoming (WY; 43° 56′N, 110°38′W), from 9 June to 19 July 2011. Observations were made between 0600 and 1200 EDT (VA) or MDT (WY). We tested 21 males in VA ($n=11$ controls, $n=10$ males exposed to long-range song), and we tested 60 males in WY ($n=20$ controls, $n=20$ males exposed to long-range song, $n=20$ males exposed to short-range song).

Both populations appear to be similar in life history, but the breeding season is notably shorter in WY (e.g. 6–8 weeks for WY versus 14–16 weeks for VA; pers. obs. DGR). While exact breeding stage was not known for most males, all males were in the early to mid breeding season at each site, as evidenced by concurrent observations of females with nesting material and incomplete to full brood patches for the first few weeks of sampling, and no observations of males caring for nestlings until the latter half of our sampling. Treatments were balanced by date and breeding stage, if known.

To ensure that playbacks and control observations did not occur near territorial boundaries, we noted the locations of song perches or nests of our subjects, and aborted any trials in which more than one male responded to the playback. We did not test neighbors on the same or consecutive days.

Observations and hormone collection from control males

We used free-living males as controls because their hormone levels should reflect the background level of social interactions encountered by free-living juncos in each population. In one population (VA) we observed behavior for 25 min prior to sampling blood for hormones by noting the number of songs sung on previously mapped territories. To capture control males from both populations we used a very brief conspecific playback (latency to capture VA [mean ± se]: 83 ± 24 s, range: 20 to 240 s; WY: 93.2 ± 20.5 s, range: 6 s to 318 s), and we collected blood very rapidly, before hormone levels were likely to have been influenced by this brief playback and/or handling (latency from capture to blood sampling: VA: 68 ± 6 s, range: 35–100 s; WY: 84.7 ± 11.8 s, range: 34 s to 232 s). We eliminated one VA male from analyses because blood sampling occurred after 4 min of handling, leaving $n=10$ controls in VA and $n=20$ controls in WY. While there is some possibility that our capture method (brief playback) may have affected hormone signaling in control males, the total time from initial disturbance to blood sampling was short (VA: 116 ± 9 s, range: 60–159 s; WY: 166 ± 22 s, range: 56–367 s), and we observed no relationship between latency to capture or bleed and any hormone measure (Pearson's $|r|<0.48$, $p>0.16$). Moreover, control hormone levels for both T and CORT fall within the normal range for baseline breeding male juncos (see results, Ketterson et al., 1991; Schoech et al., 1999), suggesting that control samples represent baseline hormone levels. In VA, each male was sacrificed with an overdose of isoflurane, followed by rapid decapitation, as a part of another experiment, and latencies to blood collection include time to sacrifice. We collected up to 300 μL trunk blood from these males directly into heparinized