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Aggressive interactions differentially modulate local and systemic levels of corticosterone and DHEA in a wild songbird

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ABSTRACT

During the nonbreeding season, when gonadal androgen synthesis is basal, recent evidence suggests that neurosteroids regulate the aggression of male song sparrows. In particular, dehydroepiandrosterone (DHEA) is rapidly converted in the brain to androgens in response to aggressive interactions. In other species, aggressive encounters increase systemic glucocorticoid levels. However, the relationship between aggression and local steroid levels is not well understood. Here, during the breeding and nonbreeding seasons, we tested the effects of a simulated territorial intrusion (STI) on DHEA and corticosterone levels in the brachial and jugular plasma. Jugular plasma is enriched with neurosteroids and provides an indirect index of brain steroid levels. Further, during the nonbreeding season, we directly measured steroid levels in the brain and peripheral tissues. Both breeding and nonbreeding males displayed robust aggressive responses to STI. During the breeding season, STI increased brachial and jugular corticosterone levels and jugular DHEA levels. During the nonbreeding season, STI did not affect plasma corticosterone levels, but increased jugular DHEA levels. During the nonbreeding season, STI did not affect brain levels of corticosterone or DHEA. However, STI did increase corticosterone and DHEA concentrations in the liver and corticosterone concentrations in the pectoral muscle. These data suggest that 1) aggressive social interactions affect neurosteroid levels in both seasons and 2) local steroid synthesis in peripheral tissues may mobilize energy reserves to fuel aggression in the nonbreeding season. Local steroid synthesis in brain, liver or muscle may serve to avoid the costs of systemic increases in corticosterone and testosterone.

Introduction

Many studies have examined the relationship between testosterone and aggression. In a wide range of taxa there is typically a positive correlation between circulating levels of testosterone and the expression of aggressive behavior across the seasons (Nelson, 1995; Simon et al., 2006). In avian species, however, the relationship between aggression and plasma testosterone levels may be particularly complex (Goymann et al., 2007). For example, breeding male European nuthatches (*Sitta europaea*) respond aggressively to a simulated territorial intrusion (STI) without a corresponding increase in circulating testosterone (Landys et al., 2010), and breeding great tits (*Parus major*) decrease circulating testosterone levels after an STI (van Duyse et al., 2004). Further, aggressive behavior can be independent of circulating testosterone levels in avian species breeding in extreme environments, such as the Arctic (Soma et al., 1999b; Wingfield and Hunt, 2002).

During the nonbreeding season, circulating testosterone levels and aggressive behavior can also be decoupled; the expression of aggressive behavior can be robust despite the regression of the gonads and basal levels of circulating testosterone. For example, the sedentary song sparrow of western North America (*Melospiza melodia morphna*) is territorial year-round. During the nonbreeding season, aggressive interactions do not increase plasma testosterone levels (Soma and Wingfield, 2001; Wingfield and Hahn, 1994) and castration does not decrease aggression in territorial males (Wingfield, 1994). Sex steroids synthesized in the brain, rather than in the gonads, might regulate aggression in song sparrows and other species outside of the breeding season (Schmidt et al., 2008; Soma et al., 2008).

Interestingly, recent studies indicate a role for the androgen precursor dehydroepiandrosterone (DHEA) in the control of aggression (Demas et al., 2007). In song sparrows, during both the breeding and nonbreeding seasons, DHEA levels are high in plasma (Soma and Wingfield, 2001; Newman et al., 2008b) and even higher in brain tissue (Newman and Soma, 2009). Several studies suggest that neurally-synthesized DHEA regulates nonbreeding aggression. For example, DHEA treatment increases territorial singing behavior in response to an STI (Soma et al., 2002) and the size of the song control nucleus HVC

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(Newman et al., 2010; Soma et al., 2002). DHEA does not bind to a classical intracellular steroid receptor (Labrie et al., 2005; Widstrom and Dillion, 2004), but DHEA is readily converted to androstenedione (AE), an active and aromatizable androgen. Activity of the enzyme that converts DHEA to AE, 3β-hydroxysteroid dehydrogenase (3β-HSD), is higher in the brain during the nonbreeding season than the breeding season, and 3β-HSD activity in portions of the forebrain is rapidly upregulated by an STI in the nonbreeding season (Pradhan et al., 2010).

In addition to DHEA, glucocorticoids also play an important role in the regulation of aggressive behavior (Mikics et al., 2004). In several species, aggressive social encounters increase circulating glucocorticoid levels (Haller et al., 1998; Summers et al., 2005). Aggressive territorial defense involves behaviors that are energetically expensive (Briffa and Sneddon, 2007; Marler et al., 1995). Glucocorticoids mobilize energy stores in fat, skeletal muscle and liver (Dallman et al., 2004; Sapolsky et al., 2000) and thus can fuel territorial behavior.

Seasonal factors clearly play an important role in the neuroendocrine regulation of territoriality. For example, circulating androgen and glucocorticoid levels change seasonally (Newman et al., 2008b; Romero, 2002), brain gene expression in response to an aggressive encounter varies seasonally (Mukai et al., 2009), and the context and functions of territorial aggression are season-dependent (Wingfield and Monk, 1992). Territorial aggression in the breeding season involves mate guarding, but territorial aggression in the nonbreeding season likely revolves around defense of food and/or refugia from predators and inclement weather. At mid- to high-latitudes, the large seasonal changes in food availability, photoperiod, and ambient temperature are likely to affect the expression and neuroendocrine regulation of aggressive behavior. For example, in autumn and winter, song sparrows must contend with low food availability, reduced daylight hours for foraging, cold temperatures, and winter storms. These factors reduce the time available for aggressive interactions and also increase the costs of high systemic testosterone and corticosterone levels (Soma, 2006).

Here, we measured the effects of a 30-min simulated territorial intrusion (STI) on local and systemic levels of corticosterone and DHEA during the breeding and nonbreeding seasons. Using non-migratory adult male song sparrows in the wild, we tested the hypothesis that aggressive interactions differentially modulate steroid concentrations in the brachial plasma (an index of systemic steroid levels) and jugular plasma (an indirect index of neural steroid levels) (Schlinger and Arnold, 1992, 1993; Newman et al., 2008b; Chin et al., 2008). Previous work suggests a role for neurally-synthesized steroids in regulating nonbreeding aggression, thus we predicted that STI would increase DHEA in jugular plasma but not in brachial plasma, particularly during the nonbreeding season. Moreover, in the nonbreeding season, we directly measured local steroid levels in the brain and peripheral organs. This is the first study to examine the effects of STI on local and systemic steroid levels across seasons.

Materials and methods

All protocols and procedures complied with institutional (University of British Columbia) and Canadian Council on Animal Care regulations.

Field protocol

Wild, adult male song sparrows in the lower mainland of British Columbia, Canada, were exposed to a simulated territorial intrusion (STI) during the mid-late breeding season (June 8–June 29) and mid-nonbreeding season (Nov 23–Dec 8) of 2005. The field protocol for these subjects has been previously described in detail (Pradhan et al., 2010). Briefly, during both seasons, subjects were exposed to 30 min of either an STI or Control (CON) treatment. STI subjects in both seasons were exposed to the same caged live male conspecific decoy, which was placed on the ground in the approximate center of the territory, along with tape-recorded conspecific song playback. The song playback included songs from several individuals, and after 10 repetitions of one song type, there was 1 min of silence before the next set of songs of a different song type (Wingfield and Hahn, 1994). Control subjects were exposed to an empty cage with no playback. Song sparrows defend territories year-round and territorial defense includes several characteristic displays. We recorded the latency to respond (call, song or flight), the latency to sing, the number of songs, the number of flights (typically directed toward the decoy), the time spent in proximity to the decoy (within 5 m), and the closest approach to the decoy. After 30 min, a mist net (that was set up ahead of time) was rapidly unfurled, and individuals were captured using a short amount of playback (breeding CON: 1.82 ± 0.49 min, $n = 10$; breeding STI: 1.89 ± 0.46 min, $n = 15$; nonbreeding CON: 1.36 ± 0.6 min, $n = 11$; nonbreeding STI: 1.64 ± 0.53 min, $n = 12$). The amount of playback used to catch subjects was similar in all 4 groups ($F_{3,47} = 0.25$, $p = 0.86$).

Blood collection

Immediately following capture, two blood samples were collected from each subject: one from the brachial vein and the other from the jugular vein. Brachial plasma is an index of systemic steroid levels, and jugular plasma is enriched with neurally-synthesized steroids (Schlinger and Arnold, 1992, 1993) and may be an indirect index of neural steroid levels. Note that acute restraint stress differentially affects brachial and jugular levels of corticosterone and DHEA (Newman et al., 2008b). The first blood collection, either from the brachial or jugular vein, was randomly assigned for each subject. During the breeding and nonbreeding seasons, both blood samples were collected within 6 min of capture (Breeding Brachial vein 1st: 2.93 ± 0.41 min; Breeding Jugular vein 1st: 2.82 ± 0.38 min; Breeding Brachial vein 2nd: 5.84 ± 0.49 min; Breeding Jugular vein 2nd: 5.89 ± 0.36 min; Nonbreeding Brachial vein 1st: 2.05 ± 0.26 min; Nonbreeding Jugular vein 1st: 2.11 ± 0.19 min; Nonbreeding Brachial vein 2nd: 3.58 ± 0.21 min; Nonbreeding Jugular vein 2nd: 3.51 ± 0.41 min). Analysis of the time required to collect the first sample by two-factor ANOVA did not reveal differences between treatment ($F_{1,54} = 0.19$, $p = 0.67$) or vein ($F_{1,54} = 0.004$, $p = 0.95$) and there was no interaction ($F_{1,54} = 0.02$, $p = 0.90$). From the brachial vein, blood was collected into heparinized micro-hematocrit tubes after puncture with a 26-gauge needle. From the jugular vein, blood was drawn into a heparinized 28-gauge fixed-needle 1 ml syringe (as in Newman et al., 2008b). Blood was kept on ice until returned to the laboratory (within 2–8 h) and then centrifuged at 10,000 rpm for 10 min. Plasma was collected and then stored at −20 °C.

Tissue collection

During the nonbreeding season only, after blood collection, individuals were euthanized by rapid decapitation (3.68 ± 0.26 min after capture). The tissue collection protocol for brain and peripheral tissues has been described in detail elsewhere (Newman and Soma, 2009; Pradhan et al., 2010). Briefly, we collected i) rostral diencephalon (rDIEN), ii) caudal diencephalon (cDIEN), iii) hippocampus (HP), iv) caudal medial nidopallium (a.k.a. "caudomedial nidopallium", NCM), a square-shaped piece of tissue below the dorsal telencephalon and hippocampus, v) dorsal telencephalon (dTEL), containing the song nucleus HVC, and vi) central medial telencephalon (cmTEL) containing the septum. All regions were collected separately from the left and right hemispheres. One half (left or right) of the rDIEN, cDIEN, HP, NCM, dTEL and cmTEL was analyzed for this study, and the other half was analyzed in a separate study of steroidogenic enzyme activity (Pradhan et al., 2010). We also collected a portion of pectoral muscle and liver in addition to the testes and adrenals. The entire dissection took <30 min. Tissues were placed into separate microcentrifuge tubes, frozen immediately on dry ice, and then transported to the laboratory and stored at −80 °C.

Body condition measurements

We measured the lengths (to the nearest 0.1 mm) of the left tarsus, left wing and cloacal protuberance (an androgen-dependent secondary sex characteristic). We also estimated abdominal and furcular fat scores (five-level visual fat index; Helms and Drury, 1960). During the nonbreeding season only, we measured the length and width of the left testis.

Steroid measurements

Steroids were extracted from tissue and plasma samples using solid-phase extraction with C_{18} columns. This procedure has been validated for song sparrows and other songbirds and provides high and consistent steroid recoveries and effectively removes interfering substances (Newman et al., 2008a; Newman and Soma, 2009; Taves et al., 2010). Briefly, tissue samples were weighed and then homogenized in ice-cold dH_2O. HPLC-grade MeOH was added, samples were vortexed, sonicated for 15 min, and shaken for 1 h, and then samples were left overnight at 4 °C. The following day, samples were shaken for 1 h and centrifuged. Next, 10 mL of dH_2O was added to the supernatants from tissue samples (or to plasma samples). Samples were loaded onto C_{18} columns (500 mg C_{18}, non-endcapped, 6 mL column volume, United Chemical Technologies, UCTCUC18156) that were in a 24-place vacuum manifold. Prior to sample loading, columns had been primed with 3 mL HPLC-grade ethanol and then equilibrated with 10 mL dH_2O. After sample loading, samples were washed with 10 mL dH_2O and then eluted with 5 mL 90% HPLC-grade MeOH, exactly as detailed previously (Newman et al., 2008a; Newman and Soma, 2009). Eluates were dried in a 40 °C water bath under a N_2 stream. Dried eluates were resuspended in 250 μL phosphate-buffered saline with gelatin that included absolute ethanol (5% of resuspension volume) (see Newman et al., 2008a).

Here, we examined recovery of 29 pg of exogenous corticosterone and 26 pg of exogenous DHEA that were added to a song sparrow plasma pool ($n=6$ replicates) and a brain tissue pool ($n=6$ replicates) prior to extraction. Corticosterone and DHEA concentrations in these samples were compared to concentrations from the same pools that did not have exogenous steroid added ($n=6$ replicates each). Recovery of corticosterone was 102.1% from plasma and 74.5% from brain, and recovery of DHEA was 76.3% from plasma and 72.8% from brain (similar to Newman and Soma, 2009).

Corticosterone and DHEA concentrations were quantified in both plasma and tissue using sensitive and specific radioimmunoassays (as in Newman and Soma, 2009). From the resuspension, 100 μL × 2 was used for the DHEA assay. For the corticosterone assay, 15 μL × 2 of the suspension was diluted to 50 μL with the phosphate-buffered diluent provided with the corticosterone radioimmunoassay. For corticosterone, intra-assay variation was 4.6%, and inter-assay variation was 5.8% (Low Control) and 12.8% (High Control). For DHEA, intra-assay variation was 6.3%, and inter-assay variation was 11.0% (Low Control) and 7.2% (High Control) ($n=6$ replicates each). For both corticosterone and DHEA, the low and high controls were provided by the manufacturers of the radioimmunoassay kits. For corticosterone, all water blanks were nondetectable (<3.12 pg/tube; $n=6$). For DHEA, the lowest point on the standard curve was 2 pg/tube, and 7 of 12 water blanks read just above the lowest standard (mean = 3.8 pg/tube) and 5 of 12 water blanks were nondetectable (<2 pg/tube).

Statistics

For corticosterone, samples with less than 3.12 pg/tube (the lowest point on the standard curve) were set to zero, and for DHEA, samples with less than 3.8 pg/tube (average of the 7 water blanks above 2 pg/tube) were set to zero. Data were corrected for recovery and transformed [$\log(x+1)$] to reduce heteroscedasticity prior to analysis with JMP IN 5.1 (SAS, Cary, NC, USA).

To evaluate the effects of season and STI on each of the 6 behaviors, we used 2-factor ANOVAs and Tukey's honestly significant difference (HSD) *post hoc* tests. Next, behavioral responses from all subjects were examined using principal component analysis (PCA), to generate a composite "aggression score." We then used a 2-factor ANOVA to evaluate the effects of season and STI on the aggression score.

For plasma, the effects of season, STI and vein (brachial or jugular) were tested using a three-factor mixed-design ANOVA, where subject identity was included in the model as a random effect, and significant effects were further investigated using Tukey's HSD *post hoc* tests. For brain, liver, muscle, gonads and adrenals, we used t-tests to test for an effect of STI during the nonbreeding season and a 1-factor ANOVA to evaluate regional differences in steroid levels within each treatment group. Further, jugular plasma levels of corticosterone and DHEA were compared to steroid levels in two brain regions (cmTEL and HP, as in Newman and Soma, 2009) using a one-factor mixed-design ANOVA, where subject identity was included as a random effect. Data are presented as means ± SEM, all tests were two-tailed, and values of $p \leq 0.05$ were considered significant.

Results

Aggressive behavior

The STI significantly increased aggressive behavior during the breeding and nonbreeding seasons (Fig. 1). Further, the magnitude of the various behavioral responses was similar in both seasons. For response latency and song latency, there was a significant interaction between season and STI treatment (response latency: $F_{1,47}=27.90$, $p<0.0001$; song latency: $F_{1,47}=111.79$, $p<0.0001$). *Post hoc* tests revealed that STI significantly decreased response and song latencies during the nonbreeding season only (Figs. 1A and B). These interactions were likely driven by the fact that basal singing (in the absence of an STI) is more frequent during the breeding season. This idea is supported by the significant interaction between season and STI treatment on the number of songs ($F_{1,47}=4.77$, $p=0.035$), and *post hoc* tests revealed that while STI treatment increased the number of songs in both seasons, control subjects sang more during the breeding season than the nonbreeding season (Fig. 1C). For the number of flights, the time spent within 5 m of the decoy, and the closest approach to the decoy, there was a significant main effect of STI treatment but no main effect of season and no interaction. The STI increased the number of flights ($F_{1,47}=39.83$, $p<0.0001$; Fig. 1D) and the time spent within 5 m ($F_{1,47}=348.67$, $p<0.0001$; Fig. 1E) and decreased the closest approach ($F_{1,47}=65.25$, $p<0.0001$; Fig. 1F).

The first PCA factor (PC1: aggression score) explained 67.3% of the total variance. We present PC1 loadings in Table 1. There was a significant interaction between season and STI on the aggression score ($F_{1,47}=10.96$, $p=0.003$). *Post hoc* tests revealed that 1) STI increased the aggression score in both the breeding and nonbreeding seasons and that 2) control subjects had a lower aggression score in the nonbreeding season than the breeding season (Fig. 2).

Body condition

Neither tarsus length nor wing length was different across seasons (wing length: $F_{1,55}=0.49$, $p=0.49$; tarsus length: $F_{1,55}=1.65$, $p=0.21$) or treatments (wing length: $F_{1,55}=0.97$, $p=0.33$; tarsus length: $F_{1,55}=1.72$, $p=0.20$) and there was no interaction. Season had a significant effect on the androgen-sensitive cloacal protuberance length (breeding: 9.98 ± 0.13 mm; nonbreeding: 4.41 ± 0.11 mm; $F_{1,55}=27.77$, $p<0.0001$) but there was no difference between treatment groups and no interaction. As expected, the cloacal protuberance length was larger

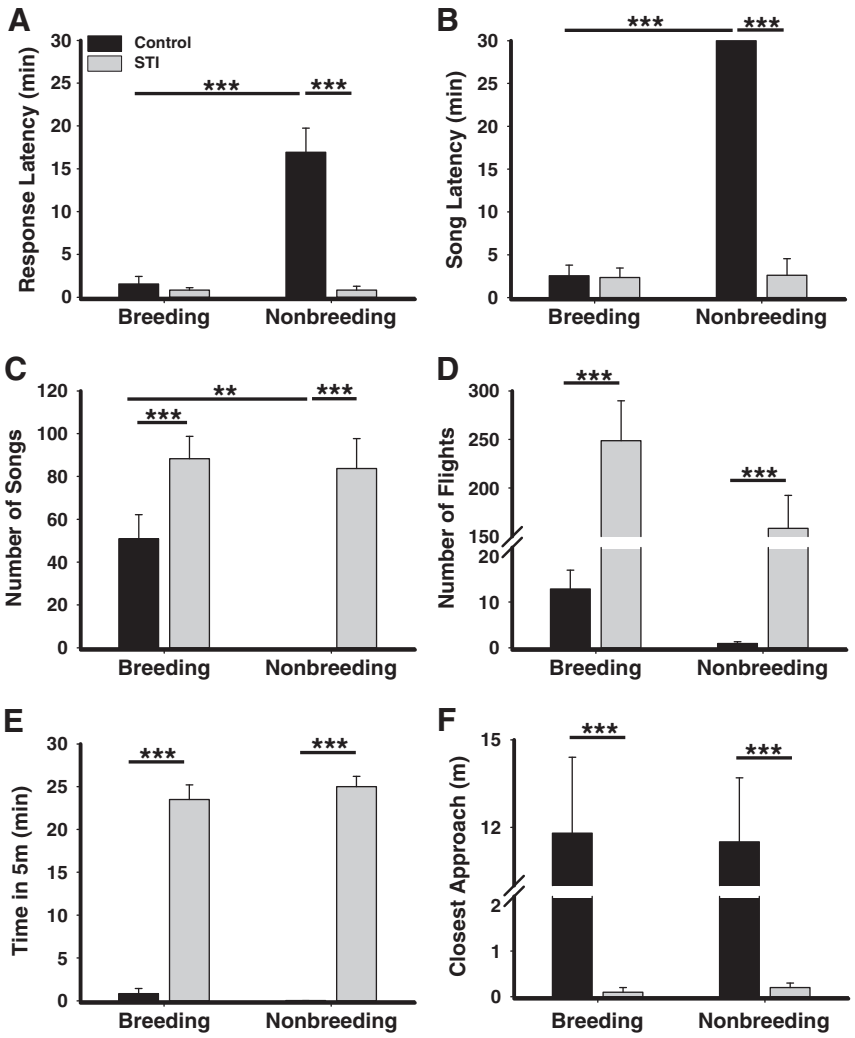

Fig. 1. Effect of a 30 min simulated territorial intrusion (STI) on aggressive responses of wild adult male song sparrows during the breeding and nonbreeding seasons. ** $p \leq 0.01$, *** $p \leq 0.0001$.

during the breeding season. Season had a significant effect on fat score (furcular + abdominal fat scores) (breeding: 0.61 ± 0.18; nonbreeding: 5.22 ± 0.21; $F_{1,55} = 259.24$, $p < 0.0001$) but there was no difference between treatment groups and no interaction. Fat scores were higher during the nonbreeding season, as in Newman et al. (2008b). In the nonbreeding season, testis volume was small (1.08 ± 0.10 mm^3), similar to previous reports (Pradhan et al., 2010; Soma et al., 2003).

Plasma steroid levels in the breeding and nonbreeding seasons

We used a 3-factor mixed-design ANOVA to examine the effects of season, STI and vein (brachial or jugular) on plasma steroid concentrations. For corticosterone, there was a significant interaction between season and treatment ($F_{1,88} = 6.78$, $p = 0.013$) but no effect of vein. *Post hoc* tests revealed that STI increased plasma corticosterone levels in both the brachial and jugular veins during the breeding season, but STI had no effect on plasma corticosterone levels in the nonbreeding season (Fig. 3A).

For DHEA, there was a significant interaction between treatment and vein ($F_{1,88} = 5.07$, $p = 0.022$) but no effect of season. *Post hoc* tests

Table 1
Principal component loadings for behavioral responses.

Component loadings	PC1
Response latency	−0.41
Song latency	−0.42
Number of songs	0.41
Number of flights	0.39
Time in 5 m	0.44
Closest approach	−0.37

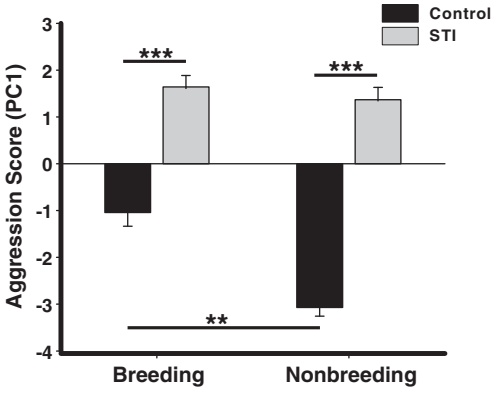

Fig. 2. Effect of STI on the aggression score (derived from principal component analysis) during the breeding and nonbreeding seasons. ** $p \leq 0.01$, *** $p \leq 0.0001$.

Table 2
Effect of STI on corticosterone concentrations (ng/g) in brain during the nonbreeding season.

	rDIEN	cDIEN	HP	NCM	dTEL	cmTEL
Control	59.4 ± 38.7	4.4 ± 2.3	9.0 ± 4.7	22.3 ± 15.0	3.0 ± 1.6	9.6 ± 3.0
	(67)	(38)	(33)	(33)	(33)	(89)
STI	11.1 ± 3.2	11.4 ± 4.9	7.1 ± 5.1	11.0 ± 4.9	8.8 ± 2.7	12.0 ± 1.5
	(78)	(67)	(25)	(44)	(67)	(100)
t-ratio	−1.2	1.3	−0.3	−0.7	1.9	0.7
p-value	0.2	0.2	0.8	0.5	0.09	0.5

Brachial plasma corticosterone level was 18.4 ± 3.5 ng/mL (n = 22). Numbers in parentheses indicate the percentage of tissue samples with detectable steroid levels. n = 8 or 9.

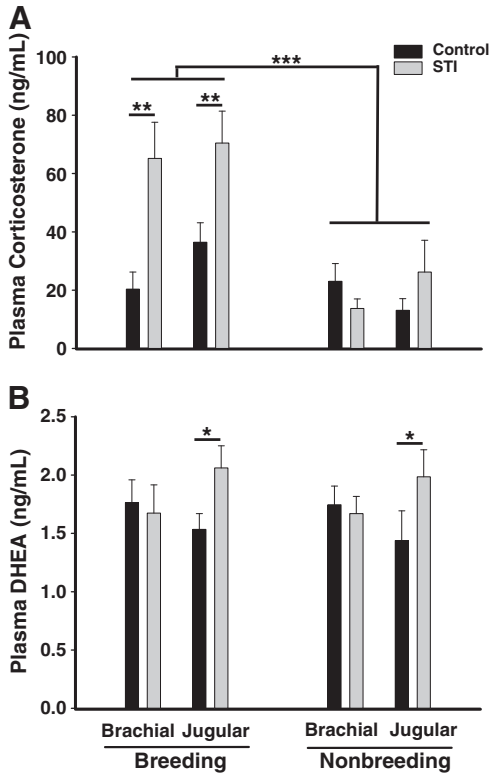

Fig. 3. Effect of STI on brachial and jugular plasma levels of corticosterone (A) and DHEA (B) during the breeding season (left) and nonbreeding season (right). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.0001$.

revealed that in both seasons, STI increased DHEA concentrations in the jugular plasma but not in the brachial plasma (Fig. 3B).

Tissue steroid levels in the nonbreeding season

As shown previously (Newman and Soma, 2009), corticosterone levels were generally greater in plasma than in brain (Fig. 3A; Table 2), while DHEA levels were generally greater in brain than in plasma (Fig. 3B; Table 3). We compared steroid concentrations in jugular plasma with those in cmTEL and HP (as in Newman and Soma, 2009). There was a significant effect of sample type (jugular plasma, cmTEL or HP) on corticosterone levels ($F_{2,56} = 3.53$, $p = 0.03$) where jugular plasma levels were higher than both cmTEL and HP levels (Tukey's HSD, $p \leq 0.05$). For DHEA levels, there was also a significant effect of sample type ($F_{2,56} = 3.33$, $p = 0.015$), however DHEA levels in jugular plasma were lower than in both cmTEL and HP (Tukey's HSD, $p \leq 0.05$).

Within each brain region, STI did not affect tissue levels of corticosterone (Table 2) or DHEA (Table 3) during the nonbreeding season. Also, there was no significant variation among brain regions in corticosterone concentrations (control: $F_{5,53} = 1.58$, $p = 0.18$; STI: $F_{5,51} = 1.42$, $p = 0.23$) or DHEA concentrations (control: $F_{5,53} = 0.80$, $p = 0.55$; STI: $F_{5,51} = 1.44$, $p = 0.23$).

We also examined the effect of STI on several peripheral tissues. STI significantly increased corticosterone levels in liver ($t = 3.79$, $p = 0.002$) and pectoral muscle ($t = 3.14$, $p = 0.007$) (Fig. 4A). STI did not affect corticosterone levels in adrenals (control: 15 196.3 ± 3341.8 ng/g, n = 8; STI: 9040.1 ± 3301.7 ng/g, n = 9; $t = -1.31$, $p = 0.21$) or gonads (control: 71.3 ± 46.98 ng/g, n = 8; STI: 123.2 ± 70.2 ng/g, n = 9, $t = 0.63$, $p = 0.46$).

For DHEA, STI increased DHEA levels in liver ($t = 2.71$, $p = 0.02$) but not in muscle ($t = 0.10$, $p = 0.9$) (Fig. 4B). Also, STI did not affect DHEA levels in adrenals (control: 30.0 ± 4.8 ng/g, n = 9; STI: 38.4 ± ng/g, n = 9; $t = 0.53$, $p = 0.60$) or gonads (control: 21.1 ± 11.5 ng/g, n = 7; STI: 32.3 ± 15.7 ng/g, n = 7; $t = 0.58$, $p = 0.57$).

Discussion

To our knowledge, this is the first report of the effects of STI on local and systemic steroid levels in different seasons. Our results suggest that aggressive interactions modulate neurally-synthesized DHEA in both the breeding and nonbreeding seasons. In both seasons, exposure to an STI rapidly increased DHEA concentrations in the jugular vein (exiting the brain) but not in the brachial vein. In stark contrast, corticosterone concentrations were similar in the jugular and brachial veins and were modulated by STI in the breeding season only. Further, during the nonbreeding season, even though systemic corticosterone levels did not increase after STI, local corticosterone concentrations in the liver and pectoral muscle did increase. These data suggest the possibility that local corticosterone synthesis in the liver and pectoral muscle mobilizes

Table 3
Effect of STI on DHEA concentrations (ng/g) in brain during the nonbreeding season.

	rDIEN	cDIEN	HP	NCM	dTEL	cmTEL
Control	3.1 ± 1.2	1.4 ± 0.6	6.7 ± 2.2	3.6 ± 1.3	2.7 ± 0.9	2.1 ± 0.5
	(100)	(89)	(100)	(89)	(67)	(100)
STI	2.3 ± 0.9	2.2 ± 0.8	4.3 ± 1.5	2.8 ± 0.9	2.9 ± 0.8	1.7 ± 0.2
	(89)	(78)	(89)	(78)	(67)	(100)
t-ratio	−0.5	0.8	−0.9	−1.1	0.2	−0.7
p-value	0.6	0.5	0.4	0.3	0.8	0.5

Brachial plasma DHEA level was 1.7 ± 0.1 ng/mL (n = 22). Numbers in parentheses indicate the percentage of tissue samples with detectable steroid levels. n = 8 or 9.

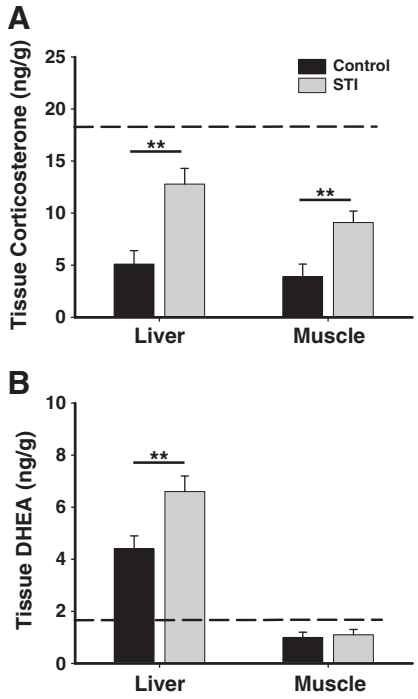

Fig. 4. Effects of STI on local levels of corticosterone (A) and DHEA (B) in liver and muscle tissue during the nonbreeding season only. Dashed lines indicate mean brachial plasma levels in all subjects during the nonbreeding season. ** $p \leq 0.01$.

energy stores to "fuel" aggressive behavior in the nonbreeding season. Such mechanisms would avoid the costs of elevated systemic levels of testosterone and corticosterone.

Aggressive response to STI

Free-living adult male song sparrows displayed robust aggressive behavior toward a live male conspecific decoy. Consistent with previous reports (Mukai et al., 2009; Soma and Wingfield, 2001; Wingfield and Hahn, 1994), the behavioral response to STI was both qualitatively and quantitatively similar during the breeding and nonbreeding seasons. This was true for each individual behavior (response latency, song latency, number of songs, number of flights, time spent within 5 m of the decoy, closest approach to the decoy). However, for non-stimulated control subjects, the aggression score was significantly greater during the breeding season. During the breeding season, control subjects sang and made a substantial number of flights on their territory, whereas control subjects did not sing during the nonbreeding season and made few flights. These results are consistent with seasonal differences in mating behavior and song control nuclei (Smith et al., 1997; Soma et al., 2004).

Effect of STI on plasma corticosterone levels

Both season and STI had dramatic effects on plasma corticosterone levels. During the breeding season only, STI increased corticosterone levels in both the brachial plasma and jugular plasma. Further, corticosterone levels in both brachial and jugular plasma were positively correlated with aggressive behavior in the breeding season only. Similar effects have been reported in the European nuthatch (Landys et al., 2010), blue tit (Landys et al., 2007; van Duyse et al., 2004) and white-crowned sparrow (Charlier et al., 2009; Lynn et al., 2007). Physical activity stimulates the hypothalamic pituitary adrenal axis (Stranahan et al., 2008) and the similarity between brachial and jugular levels suggests a global increase in corticosterone to mobilize energy stores to meet the demands of territorial and mate defense. In stitchbirds (*Notiomystis cincta*), there are clear energetic costs (weight loss) to resident males when exposed to frequent conspecific territorial intrusions during the breeding season (Low, 2006).

In contrast, during the nonbreeding season, there was no effect of STI on brachial or jugular plasma levels of corticosterone. However, a 30 min restraint stress during the nonbreeding season elicits a significant increase in both brachial and jugular corticosterone levels (Newman et al., 2008b; Newman and Soma, 2009), thus demonstrating that the adrenals remain active during the nonbreeding season. It is possible that the costs of elevated systemic corticosterone concentrations are higher during the nonbreeding season, resulting instead in local corticosterone synthesis to mobilize energy stores (see below) or mediate the inflammatory effects of acute physical activity (Taves et al., 2011). For example, high circulating levels of corticosterone can suppress immune function (Martin et al., 2005). Alternatively, there may be seasonal differences at the level of glucocorticoid receptors or corticosterone binding globulin (CBG) (Breuner and Orchinik, 2001). During the breeding season, STI increases plasma CBG in white-crowned sparrows (Charlier et al., 2009), however the effect of STI on CBG during the nonbreeding season is unknown. Lastly, the context of a territorial intrusion may differ across seasons, resulting in different physiological responses, depending on the subject's perception of the threat.

Effect of STI on plasma DHEA levels

There was no main effect of season on plasma DHEA levels, but there was a vein × STI interaction where STI increased DHEA levels in jugular plasma but not in brachial plasma. Importantly, it is evident that the regulation of plasma DHEA levels is stimulus-specific. In contrast to the present data, a 30 min restraint stress decreases jugular DHEA levels during the breeding season and has no effect on jugular DHEA levels during the nonbreeding season (Newman et al., 2008b; Newman and Soma, 2009). A lack of STI effect on brachial DHEA levels is consistent with a previous study (Soma and Wingfield, 2001).

An increase in jugular DHEA levels suggests an increase in neural DHEA synthesis and/or a decrease in neural DHEA metabolism. However, there is clear evidence that neural DHEA metabolism increases following STI in the nonbreeding season (Pradhan et al., 2010), arguing against the latter possibility. Thus, taken together, the data suggest an increase in both neural DHEA synthesis (present study) and neural DHEA metabolism (Pradhan et al., 2010) following STI. Note that Wingfield and Hahn (1994) found a slight, but non-significant, increase in brachial testosterone levels after STI in this song sparrow subspecies (*M. m. morphna*) during the breeding season, which further highlights the significant increase in jugular DHEA seen here.

The enzymes that synthesize DHEA may be expressed in the brain year-round. Thus, despite the previous focus on neurosteroid regulation of nonbreeding behavior (Soma et al., 2008), neurosteroids may also facilitate territorial behavior during the breeding season. This possibility would greatly expand the potential roles of neurosteroids throughout the various stages of the life history cycle.

Effect of STI on brain corticosterone and DHEA levels during the nonbreeding season

Brain levels of corticosterone were lower than plasma levels, whereas brain levels of DHEA were higher than plasma levels (as in Newman and Soma, 2009). Neither corticosterone nor DHEA levels in the brain were affected by STI. However, there is evidence that the enzymes that metabolize DHEA to active androgens and estrogens (3β-HSD, aromatase) regulate aggression during the nonbreeding season (Pradhan et al., 2010; Soma et al., 1999a,b; Soma et al., 2000a,b). Thus, we expected STI to modulate brain levels of DHEA. However, as mentioned above, an increase in neural DHEA synthesis coincident with an increase in neural DHEA metabolism would yield no net change in

neural DHEA concentrations. For example, the androgen synthetic enzyme, cytochrome P450c17 (CYP17), synthesizes DHEA and is expressed in the songbird brain (Schlinger and London, 2006). STI might increase activity of CYP17 or an upstream steroidogenic enzyme. Lastly, neurosteroids can passively diffuse or be actively transported out of tissue and into the bloodstream (Pariante, 2008), and thus an increase in neural DHEA synthesis could increase DHEA levels "escaping" from the brain into the jugular blood.

Alternatively, neurosteroids and enzymatic activity can fluctuate rapidly (Balthazart and Ball, 2006; Cornil et al., 2005; Remage-Healey et al., 2008), and the 30 min STI used here may have been too long to detect a change in neurosteroid levels. Future studies should examine the effects of STI at earlier time-points (e.g., 5 or 10 min; Croft et al., 2008).

Effect of STI on peripheral tissues

Exposure to an STI during the nonbreeding season increased corticosterone concentrations in both the liver and muscle, but not in plasma, suggesting local corticosterone synthesis or sequestration. Total corticosterone concentrations were lower in liver and muscle than in plasma, but this observation by itself cannot exclude the possibility of local corticosterone synthesis in these tissues. First, it is likely that corticosterone measured in tissue is primarily unbound (free), while corticosterone measured in plasma is primarily (over 90%) bound to CBG and cannot readily enter tissues (Breuner and Orchinik, 2002). Second, levels of corticosterone are approximately twice as high in plasma as in whole blood, and thus plasma samples overestimate circulating levels of corticosterone in the organism (Taves et al., 2010).

Glucocorticoids can increase blood glucose levels by promoting the breakdown of glycogen, and they also promote the breakdown of triglycerides (Remage-Healey and Romero, 2001), particularly in the liver and muscle (Leung and Munck, 1975). Interestingly, during the nonbreeding season, liver and muscle glycogen stores increase in dark-eyed juncos (Swanson, 1991), and the liver mass of male song sparrows significantly increases from 0.997 ± 0.057 g in the breeding season to 1.393 ± 0.037 g in the nonbreeding season ($t = 5.23$, $p < 0.0001$; K. Soma, unpublished data). Several studies suggest that hepatic and muscle glycogen stores are sources of energy during aggressive encounters. In male rats and mice, liver and muscle glycogen levels decrease after an aggressive interaction with a conspecific (Haller, 1993; Sanchez et al., 2007). In male Coho salmon, subordinate individuals, who are subject to frequent aggression by dominant conspecifics, have elevated circulating glucocorticoids and reduced hepatic glycogen levels (Ejike and Schreck, 1980). Further, in skeletal muscle, physical activity upregulates activity of 11β-hydroxysteroid dehydrogenase type 1, which regenerates active glucocorticoids from inactive metabolites (Coutinho et al., 2006). During the nonbreeding season, control subjects were less active than STI subjects, which made ~150 flights. Also, during the nonbreeding season, within the daily energy budget, the calories required per hour are highest for territorial defense in several avian species (Davies, 1980). Thus, physical activity may stimulate local steroid synthesis in liver and muscle tissue, and local increases in glucocorticoids may regulate energy mobilization during acute periods of increased activity (e.g., territorial defense) while avoiding the costs of systemic glucocorticoids.

Elevated corticosterone levels, however, have detrimental side effects in the liver. Social stress (isolation or confrontation) increases plasma glucocorticoid levels and liver metastasis (Wu et al., 2000). Further, male mice exposed to a 30 min aggressive encounter had elevated plasma corticosterone levels and significant damage to the liver (Sanchez et al., 2007). Here, STI exposure also increased DHEA concentrations in liver, but not in brachial plasma. The liver expresses the enzymes required to synthesize DHEA de novo (Grasfeder et al., 2009; Vianello et al., 1997). DHEA is a potent antiglucocorticoid in the liver (Kalimi et al., 1994) and may counteract some effects of elevated local corticosterone levels. Evidence suggests that DHEA regulates liver metabolism, particularly during fasting (Bauer et al., 2004; Grasfeder et al., 2009), and might act to maintain the balance between energy mobilization and glucocorticoid-induced tissue damage. Similar protective effects of elevated DHEA have been observed in the brain of adult male song sparrows treated with corticosterone implants (Newman et al., 2010).

Conclusions

Several studies have examined the neuroendocrine mechanisms regulating aggression in the nonbreeding season, when gonadal steroid production is basal. While neural DHEA metabolism and estradiol synthesis have been previously implicated in maintaining nonbreeding aggression, these results are the first to suggest a role for neurally-synthesized DHEA in the breeding season as well. Further, during the nonbreeding season, aggressive encounters affect the local steroid environments in the liver and pectoral muscle. Both corticosterone and DHEA levels increased in liver after STI exposure, despite a lack of change in systemic levels of either steroid. Together, these data suggest multiple roles for local steroid synthesis in the regulation of aggressive behavior, possibly to avoid the costs associated with systemic increases in circulating glucocorticoids and androgens.

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References

Balthazart, J., Ball, G.F., 2006. Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci. 29, 241–249.
Bauer, M., Hamm, A.C., Bonaus, M., Jacob, A., Jaekel, J., Schorle, H., Pankratz, M.J., Katzenberger, J.D., 2004. Starvation response in mouse liver shows strong correlation with life-span-prolonging processes. Physiol. Genomics 17, 230–244.
Breuner, C.W., Orchinik, M., 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the House Sparrow brain. J. Neuroendocrinol. 13, 412–420.
Breuner, C.W., Orchinik, M., 2002. Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.
Briffa, M., Sneddon, L.U., 2007. Physiological constraints on contest behavior. Funct. Ecol. 21, 627–637.
Charlier, T.D., Underhill, C., Hammond, G.L., Soma, K.K., 2009. Effects of aggressive encounters on plasma corticosteroid-binding globulin and its ligands in white-crowned sparrows. Horm. Behav. 56, 339–347.
Chin, E.H., Shah, A.H., Schmidt, K.L., Sheldon, L.D., Love, O.P., Soma, K.K., 2008. Sex differences in DHEA and estradiol during development in a wild songbird: jugular versus brachial plasma. Horm. Behav. 54, 194–202.
Cornil, C.A., Ball, G.F., Balthazart, J., 2005. Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? Brain Res. 1126, 2–26.
Coutinho, A.E., Campbell, J.E., Fediuc, S., Riddell, M.C., 2006. Effect of voluntary exercise on peripheral tissue glucocorticoid receptor content and the expression and activity of 11β-HSD1 in the Syrian hamster. J. Appl. Physiol. 100, 1483–1488.
Croft, A.P., O'Callaghan, M.J., Shaw, S.G., Connolly, G., Jacquot, C., Little, H.J., 2008. Effects of minor laboratory procedures, adrenalectomy, social defeat or acute alcohol on regional brain concentrations of corticosterone. Brain Res. 1238, 12–22.
Dallman, M.F., la Fleur, S.E., Pecoraro, N.C., Gomez, F., Houshyar, H., Akana, S.F., 2004. Minireview: glucocorticoids, food intake, abdominal obesity, and wealthy nations in 2004. Endocrinology 145, 2633–2638.
Davies, N.B., 1980. The economics of territorial behavior in birds. Ardea 68, 63–74.
Demas, G.E., Cooper, M.A., Albers, H.E., Soma, K.K., 2007. Novel mechanisms underlying neuroendocrine regulation of aggression: a synthesis of rodent, avian, and primate studies. In: Blaustein, J.D. (Ed.) Behavioral Neurochemistry and Neuroendocrinology. In: Lajtha, A. (Ed.), Handbook of Neurochemistry and Molecular Neurobiology, vol. 21. Springer Science + Business Media, LLC, New York, pp. 337–372.

Ejike, C., Schreck, C.B., 1980. Stress and social hierarchy rank in Coho salmon. Trans. Am. Fish. Soc. 109, 423–426.
Goymann, W., Landys, M.M., Wingfield, J.C., 2007. Distinguishing seasonal androgen responses from male-male androgen responsiveness-Revisiting the challenge hypothesis. Horm. Behav. 51, 463–476.
Grasfeder, L.L., Gaillard, S., Hammes, S.R., Ilkayeva, O., Newgard, C.B., Hochberg, R.B., Dwyer, M.A., Chang, C., McDonnell, D.P., 2009. Fasting-induced hepatic production of DHEA is regulated by PGC-1α, ERRα, HNF4α. Mol. Endocrinol. 23, 1171–1182.
Haller, J., 1993. Adrenomedullar catecholamine liberation and carbohydrate metabolism during the first 30 min of an aggressive encounter in rats. Physiol. Behav. 54, 195–197.
Haller, J., Halasz, J., Makara, G.B., Kruk, M.R., 1998. Acute effects of glucocorticoids: behavioral and pharmacological perspectives. Neurosci. Biobehav. Rev. 23, 337–344.
Helms, C.W., Drury, W.H., 1960. Winter and migratory weight and fat. Field studies on some North American buntings. Bird Banding 31, 1–40.
Kalimi, M., Shafagoj, Y., Loria, R., Padgett, D., Regelson, W., 1994. Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA). Mol. Cell. Biochem. 131, 99–104.
Labrie, F., Luu-The, V., Belanger, A., Lin, S.X., Simard, J., Pelletier, G., Labrie, C., 2005. Is dehydroepiandrosterone a hormone? J. Endocrinol. 187, 169–196.
Landys, M.M., Goymann, W., Raess, M., Slagsvold, T., 2007. Hormonal responses to male–male social challenge in the Blue Tit *Cyanistes caeruleus*: single broodedness as an explanatory variable. Physiol. Biochem. Zool. 80, 228–240.
Landys, M.M., Goymann, W., Schwabl, I., Trapschuh, M., Slagsvold, T., 2010. Impact of season and social challenge on testosterone and corticosterone in a year-round territorial bird. Horm. Behav. 58, 317–325.
Leung, K., Munck, A., 1975. Peripheral actions of glucocorticoids. Annu. Rev. Physiol. 37, 245–272.
Low, M., 2006. The energetic cost of mate guarding is correlated with territorial intrusions in the New Zealand stitchbird. Behav. Ecol. 17, 270–276.
Lynn, S.E., Hahn, T.P., Breuner, C.W., 2007. Free-living male mountain white-crowned sparrows exhibit territorial aggression without modulating total or free plasma testosterone. Condor 109, 173–180.
Marler, C.A., Walsberg, G., White, M.L., Moore, M., 1995. Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. Behav. Ecol. Sociobiol. 37, 225–231.
Martin, L.B., Gilliam, J., Han, P., Lee, K., Wikelski, M., 2005. Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus*. Gen. Comp. Endocrinol. 140, 126–135.
Mikics, E., Kruk, M.R., Haller, J., 2004. Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. Psychoneuroendocrinology 29, 618–635.
Mukai, M., Replogle, K., Drnevich, J., Wang, G., Wacker, D., Band, M., Clayton, D.F., Wingfield, J.C., 2009. Seasonal differences of gene expression profiles in song sparrow (*Melospiza melodia*) hypothalamus in relation to territorial aggression. PLoS One 4, 1–14.
Nelson, R.J., 1995. An introduction to Behavioral Endocrinology. Sinaur Assoc., Sunderland, MA.
Newman, A.E.M., Soma, K.K., 2009. Corticosterone and dehydroepiandrosterone in songbird sparrow plasma and brain: effects of season and acute stress. Eur. J. Neurosci. 29, 1905–1914.
Newman, A.E.M., Chin, E.H., Schmidt, K.L., Bond, L., Wynne-Edwards, K.E., Soma, K.K., 2008a. Analysis of steroids in songbird plasma and brain by coupling solid-phase extraction to radioimmunoassay. Gen. Comp. Endocrinol. 155, 503–510.
Newman, A.E.M., Pradhan, D.S., Soma, K.K., 2008b. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular *versus* brachial plasma. Endocrinology 149, 2537–2545.
Newman, A.E.M., MacDougall-Shackleton, S.A., An, Y.-S., Kriengwatana, B., Soma, K.K., 2010. Corticosterone and dehydroepiandrosterone have opposing effects on adult neuroplasticity in the avian song control system. J. Comp. Neurol. 518, 3662–3678.
Pariante, C.M., 2008. The role of multi-drug resistance p-glycoprotein in glucocorticoid function: studies in animals and relevance in humans. Eur. J. Pharmacol. 583, 263–271.
Pradhan, D.S., Newman, A.E.M., Wacker, D.W., Wingfield, J.C., Schlinger, B.A., Soma, K.K., 2010. Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. Horm. Behav. 57, 381–389.
Remage-Healey, L., Romero, L.M., 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281, R994–R1003.
Remage-Healey, L., Maidment, N.T., Schlinger, B.A., 2008. Forebrain steroid levels fluctuate rapidly during social interactions. Nat. Neurosci. 11, 1327–1334.
Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.
Sanchez, O., Vildarich, M., Ramirez, I., Soley, M., 2007. Liver injury after an aggressive encounter in mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1908–R1916.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive stimulatory and preparative actions. Endocr. Rev. 21, 55–89.
Schlinger, B.A., Arnold, A.P., 1992. Circulating estrogens in a male songbird originate in the brain. Proc. Natl. Acad. Sci. USA 89, 7650–7653.
Schlinger, B.A., Arnold, A.P., 1993. Estrogen synthesis *in vivo* in the adult zebra finch: additional evidence that circulating estrogens can originate in brain. Endocrinology 133, 2610–2616.
Schlinger, B.A., London, S.E., 2006. Neurosteroids and the songbird model system. J. Exp. Zool. 305A, 743–748.
Schmidt, K.L., Pradhan, D.S., Shah, A.H., Charlier, T.D., Chin, E.H., Soma, K.K., 2008. Neurosteroids, immunosteroids, and the Balkanization of endocrinology. Gen. Comp. Endocrinol. 157, 266–274.
Simon, N.G., Mo, Q., Hu, S., Garippa, C., Lu, S., 2006. Hormonal pathways regulating intermale and interfemale aggression. Int. Rev. Neurobiol. 73, 99–123.
Smith, G.T., Brenowitz, E.A., Beecher, M.D., Wingfield, J.C., 1997. Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. J. Neurosci. 17, 6001–6010.
Soma, K.K., 2006. Testosterone and aggression: Berthold, birds and beyond. J. Neuroendocrinol. 18, 543–551.
Soma, K.K., Wingfield, J.C., 2001. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. Gen. Comp. Endocrinol. 123, 144–155.
Soma, K.K., Bindra, R.K., Gee, J., Wingfield, J.C., Schlinger, B.A., 1999a. Androgen metabolizing enzymes show region-specific changes across the breeding season in the brain of a wild songbird. J. Neurobiol. 41, 176–188.
Soma, K.K., Sullivan, K., Wingfield, J.C., 1999b. Combined aromatase inhibitor and antiandrogen treatment decreases territorial aggression in a wild songbird during the nonbreeding season. Gen. Comp. Endocrinol. 115, 442–453.
Soma, K.K., Sullivan, K., Tramontin, A.D., Saldanha, C.J., Schlinger, B.A., Wingfield, J.C., 2000a. Acute and chronic effects of an aromatase inhibitor on territorial aggression in breeding and nonbreeding male song sparrows. J. Comp. Physiol. [A] 186, 759–769.
Soma, K.K., Tramontin, A.D., Wingfield, J.C., 2000b. Oestrogen regulates male aggression in the non-breeding season. Proc. Biol. Sci. B 267, 1089–1096.
Soma, K.K., Wissman, A.M., Brenowitz, E.A., Wingfield, J.C., 2002. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm. Behav. 41, 203–212.
Soma, K.K., Schlinger, B.A., Wingfield, J.C., Saldanha, C.J., 2003. Brain aromatase, 5α-reductase, and 5β-reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior. J. Neurobiol. 56, 209–221.
Soma, K.K., Tramontin, A.D., Featherstone, J., Brenowitz, E.A., 2004. Estrogen contributes to seasonal plasticity of the adult avian song control system. J. Neurobiol. 15, 413–422.
Soma, K.K., Scotti, M.L., Newman, A.E.M., Charlier, T.D., Demas, G.E., 2008. Novel mechanisms for neuroendocrine regulation of aggression. Front. Neuroendocrinol. 29, 476–489.
Stranahan, A.M., Lee, K., Mattson, M.P., 2008. Central mechanisms of HPA axis regulation by voluntary exercise. Neuromolecular Med. 10, 118–127.
Summers, C.H., Watt, M.J., Link, T.L., Forster, G.L., Carpenter, R.E., Korzan, W.J., Lukkes, J.L., Oyvind, O., 2005. Glucocorticoid interaction with aggression in non-mammalian vertebrates: reciprocal action. Eur. J. Pharmacol. 526, 21–35.
Swanson, D.L., 1991. Substrate metabolism under cold stress in seasonally acclimatized dark-eyed juncos. Physiol. Zool. 64, 1578–1592.
Taves, M.D., Schmidt, K.L., Ruhr, I.M., Kapusta, K., Prior, N.H., Soma, K.K., 2010. Steroid concentrations in plasma, whole blood, and brain: effects of saline perfusion to remove blood contamination from brain. PLoS One 5, e15727.
Taves, M.D., Gomez-Sanchez, C.E., Soma, K.K., 2011. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. Am. J. Physiol. Endocrinol. Metab. 301, E11–E24.
Van Duyse, E., Pinxten, R., Darras, V.M., Arckens, L., Eens, M., 2004. Opposite changes in plasma testosterone and corticosterone levels following a simulated territorial challenge in male great tits. Behaviour 141, 451–467.
Vianello, S., Waterman, M.R., Dalla Valle, L., Colombo, L., 1997. Developmentally regulated expression and activity of 17 alpha-hydroxylase/C-17,20-lyase cytochrome P450 in rat liver. Endocrinology 138, 3166–3174.
Widstrom, R.L., Dillion, J.S., 2004. Is there a receptor for dehydroepiandrosterone or dehydroepiandrosterone sulfate. Semin. Reprod. Med. 22, 289–298.
Wingfield, J.C., 1994. Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. Horm. Behav. 28, 1–15.
Wingfield, J.C., Hahn, T.P., 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Anim. Behav. 47, 77–89.
Wingfield, J.C., Hunt, K.E., 2002. Arctic spring: hormone-behavior interactions in a severe environment. Comp. Biochem. Physiol. B 132, 275–286.
Wingfield, J.C., Monk, D., 1992. Control and context of year-round territorial aggression in the non-migratory song sparrow *Zonotrichia melodia morphna*. Ornis Scand. 23, 298–303.
Wu, W.J., Yamaura, T., Murakami, K., Murata, J., Matsumoto, K., Watanabe, H., Saiki, I., 2000. Social isolation stress enhanced liver metastasis of murine colon 26-L5 carcinoma cells by suppressing immune responses in mice. Life Sci. 66, 1827–1838.