

Chapter 11

Brain Aromatase and Territorial Aggression Across the Seasons in Male Song Sparrows

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INTRODUCTION

Estrogens regulate a variety of social behaviors, including appetitive and consummatory sexual behavior, social affiliation, and aggression. Much of our understanding of the roles of estrogens in social behavior comes from laboratory studies of domesticated mice and rats (Blumstein et al., 2010; Soma et al., 2008). In addition to this work, field studies of free-living animals offer the opportunity to investigate how estrogens regulate naturally occurring social behaviors within an ecologically relevant context. Such work is important because it has provided novel insights and perspectives into the estrogenic regulation of complex social behaviors and has been critical to developing a comprehensive understanding of the variety of contexts in which estrogens regulate social behavior. One such example is research on the role of 17β -estradiol (E_2) in regulating male song sparrow (*Melospiza melodia*) territorial aggression in different seasons.

Our understanding of songbird natural history, behavior, social patterning, and seasonal endocrine physiology is far greater than for most vertebrates. Song sparrows played a critical role in the formulation of principles about testosterone and aggression, such as the Challenge Hypothesis described by Wingfield et al. (1990). Further, the songbird brain expresses the estrogen-synthetic enzyme aromatase more widely and in higher abundance than nonsongbird species, including rodents (Forlano et al., 2006). Moreover, songbird behavior and adult neuroplasticity are highly estrogen sensitive and seasonally variable (Ball et al., 2004; Soma, 2006; Schlinger et al., 2008). Together, these factors make songbirds outstanding model systems for studying estrogenic regulation of complex social behaviors.

ECOLOGICAL, SOCIAL, AND BEHAVIORAL CONTEXT OF TERRITORIALITY

Song sparrows are common and have a widespread distribution throughout North America. Populations in northern and eastern North America are typically long distance migrants to avoid the severe winter conditions in these regions (Arcese et al., 2002). In contrast, western populations along the Pacific

Table 11–1. Seasonal Changes in Daylength, Temperature, and Precipitation Near Vancouver, BC (49°12'N, 123°01'W)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Daylength (hr:min)*	9:18	10:31	11:53	13:26	14:43	15:25	15:07	13:59	12:30	11:02	9:40	8:57
Mean temperature (°C)	3.6	4.9	6.6	9.1	12.3	14.7	16.9	17.1	14.5	10.3	6.1	3.8
Max. temperature (°C)	6	7.6	9.5	12.3	15.7	18	20.4	20.5	17.9	13.1	8.5	6.1
Min. temperature (°C)	1.2	2.3	3.6	5.7	8.8	11.4	13.2	13.5	11.1	7.5	3.7	1.5
Rainfall (mm)	146.5	125.2	118.7	89	68.3	55.5	39.3	48.1	58.6	113.3	196.1	167.9

*Daylength for day in middle of each month.

1971–2010 Canadian Weather Service (Environment Canada), Delta, British Columbia, Canada.

coast experience less severe winter climates with relatively moderate temperatures and little to no persistent snow cover (Table 11–1) (Arcese et al., 2002). As a result, song sparrows endemic to this region typically do not migrate or flock, but rather are sedentary and exhibit year-round territoriality (Arcese, 1989; Wingfield & Monk, 1992). For the western populations, territorial behavior in the autumn and winter (nonbreeding season) plays an important role in over-winter survival (Smith et al., 1980; Rogers et al., 1991).

Despite the relatively moderate winter conditions, low nightly temperatures have large metabolic costs for these small songbirds (23–25 g body mass) with a high body temperature (41°C) (Rogers et al., 1991; Rogers, 1995). For example, in captive male western song sparrows exposed to a short photoperiod (8L:16D), subjects exposed to an 8°C overnight temperature exhibit greater declines in body mass and fat stores than subjects exposed to a 15°C overnight temperature (Fig. 11–1) (Soma & Wingfield, unpublished data). Subjects exposed to an 8°C overnight temperature lost nearly 9% of their body mass in one night, and nightly temperatures in winter are often below 8°C. Thus, a central function of nonbreeding territoriality is likely resource (food) defense. It is critical to secure access to food so as to meet the large energetic costs of over-wintering at northern latitudes (Wingfield & Soma, 2002).

Breeding territories are defended by a male–female pair. In contrast, at some locations, only 30% of nonbreeding territories are defended by a male–female pair (Wingfield & Soma, 2002). Nonbreeding territories can also be defended by single birds, same-sex pairs, or associations of three or more birds. The majority of nonbreeding territory holders are adults that previously bred, but

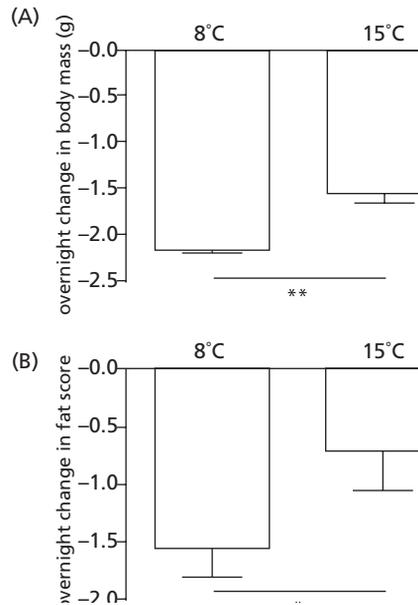


Figure 11–1. Overnight Change in (A) Body Mass and (B) Fat Score in Captive Adult Male Song Sparrows Housed at 8°C. or 15°C. Subjects were in nonbreeding condition and were housed under a 8L:16D photoperiod. Abdominal and furcular fat reserves were each scored on a five-level visual fat index (Helms & Drury, 1960) and then these two scores were summed for each individual. Note that over a single night, subjects at 8°C lost approximately 8.9% of their body mass, and subjects at 15°C lost approximately 6.4% of their body mass. K. Soma and J. Wingfield (unpublished data). * $p \leq .05$; ** $p \leq .01$, *** $p \leq .001$.

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many juveniles also establish territories in autumn (Arcese, 1987). In some cases, there is little spatial overlap between breeding and nonbreeding territories (Wingfield & Monk, 1992) (but see Arcese, 1987, 1989).

Song sparrows have been extensively used to study aggression during territorial disputes (Nowicki et al., 1998, 2002; Anderson et al., 2008a,b). When confronted with an intruder, a territorial male exhibits stereotypical vocal and display behaviors (Nice, 1943; Elekonich, 2000). These behaviors are easily elicited and quantified in the field in response to a simulated territorial intrusion (STI). An STI consists of placing a caged live decoy (or mount) in the approximate center of a territory along with tape-recorded conspecific song playback (Wingfield, 1985). The latency to respond, latency to sing, number of songs, number of flights (typically directed toward the decoy), time spent within 5 meters of the decoy, and closest approach to the decoy are then recorded. These behaviors are considered indicators of aggressiveness in this context. An STI typically lasts 10 to 30 minutes, depending on the experiment. Behavior can also be monitored after the STI (with the decoy removed and song playback

turned off), to assess the persistence of the territorial response. This behavioral testing paradigm has provided much information about songbird territorial behavior and the roles of hormones, particularly steroids, in territorial aggression (Wingfield & Monk, 1992; Wingfield, 1994c).

In sedentary song sparrow populations, aggressive behavior during an STI is qualitatively and quantitatively similar in the breeding and nonbreeding seasons (Wingfield & Hahn, 1994; Mukai et al., 2009; Newman & Soma, 2011). *During* an STI, breeding and nonbreeding males exhibit similar levels of singing behavior, flights directed at the decoy, time spent within 5 meters, and closest approach to the decoy (Fig. 11–2) (Newman & Soma, 2011). However, *before* and *after* an STI, there are some seasonal differences in aggressive behavior. Breeding males exhibit higher baseline territorial singing behavior before an STI (see Fig. 11–2) and higher persistence of singing behavior after an STI

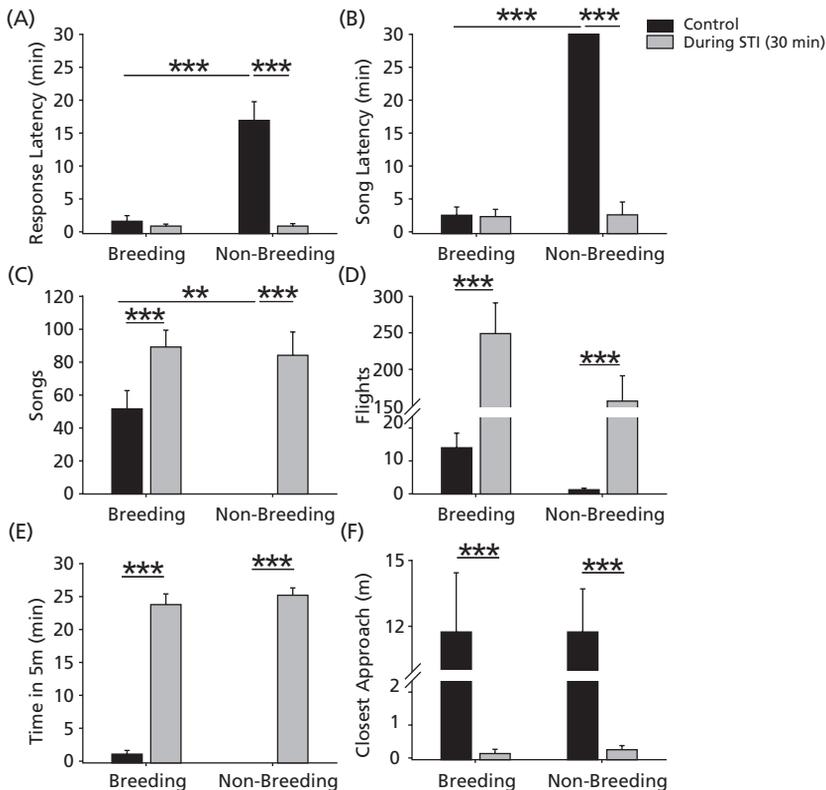


Figure 11–2. Effect of a Simulated Territorial Intrusion for 30 Minutes on Aggressive Responses of Wild Adult Male Song Sparrows During the Breeding and Nonbreeding Seasons. $^{**}p \leq .01$, $^{***}p \leq .0001$. Reprinted with permission from Newman, A. E., & Soma, K. K. (2009). Corticosterone and dehydroepiandrosterone in songbird plasma and brain: effects of season and acute stress. *Eur J Neurosci*, 29(9), 1905–1914.

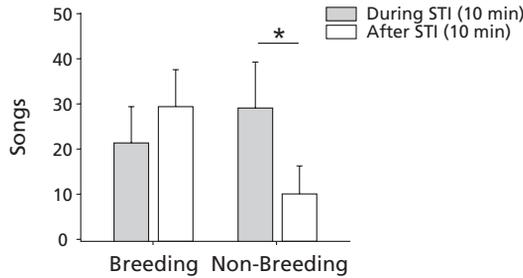


Figure 11–3. Number of Songs Given by Wild Adult Male Song Sparrows During (10 Minutes) and After (10 Minutes) a Simulated Territorial Intrusion in the Breeding and Nonbreeding Seasons. $*p < .05$. Redrawn from data published in Soma, K. K., Sullivan, K. A., Tramontin, A. D., Saldanha, C. J., Schlinger, 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(7–8), 759–769; 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*, 58(3), 413–422.

is terminated (Fig. 11–3) (Wingfield, 1994b; Newman & Soma, 2011). One explanation for this seasonal plasticity in the persistence of aggression is that the function and context of aggressive behavior varies across different seasons. Breeding aggression primarily involves monopolizing access to a female mate to avoid cuckoldry, which may require continued vigilance even after an intruder has apparently disappeared (Wingfield, 1994b). Nonbreeding aggression primarily involves monopolizing food resources. Persistent nonbreeding aggression would be disadvantageous because it would reduce the amount of time available for foraging, which is already difficult because of shorter day lengths, inclement weather, and decreased food availability (Wingfield, 1994b).

Also, note that territorial aggression is dramatically reduced during the annual molt (August to September), when song sparrows lose their old feathers and grow new ones. Aggression might be reduced during the molt because agonistic encounters that escalate to fights might damage the fragile new feathers (Wingfield, 1994a). Alternatively, molting song sparrows cannot fly and evade predators very well, and they might reduce conspicuous behaviors (e.g., singing and other territorial behaviors) to avoid detection by predators.

SEASONAL CHANGES IN PHYSIOLOGY AND MORPHOLOGY

Although territorial aggression during an STI is similar in the breeding and non-breeding seasons, the birds are in very different physiological conditions in each season. As with most songbirds living at temperate latitudes, the increasing day-lengths typical of the early breeding season (March and April) (see Table 11–1) initiates gonadal recrudescence in song sparrows. In males, this increase in testes size is accompanied by elevated levels of circulating testosterone, which

dramatically affect peripheral morphology, including decreased body mass and fat stores and increased cloacal protuberance length (used for sperm storage and delivery) (Table 11–2). After the breeding season ends, the gonads regress, circulating testosterone levels fall, body mass and fat stores increase, and the cloacal protuberance length decreases (see Table 11–2). Some of these seasonal changes in reproductive physiology (gonad size, plasma testosterone levels) are very pronounced, yet the territorial behavior remains similar.

Plasma testosterone levels also affect the brain, including various aspects of the chemical neuroanatomy of the song control system. This is a circuit of discrete brain nuclei dedicated to song learning and song production. For example, the volumes of HVC (abbreviation used as a proper name) and robust nucleus of the arcopallium (RA) are significantly smaller in nonbreeding than breeding male song sparrows (Smith et al., 1997). Both of these song nuclei contain androgen receptors, and HVC also expresses estrogen receptors (Bernard et al., 1999; Wacker et al., 2010). This is one of the best examples of naturally

Table 11–2. Seasonal Changes in Morphology and Plasma Steroid Hormones in Male Song Sparrows

	Breeding	Nonbreeding	Reference
Body mass (g)	23.32 + 1.22	25.75 + 1.86	Newman & Soma, unpublished
Fat score (furcular + abdominal)*	0.61 + 0.18	5.22 + 0.21	Newman & Soma (2011)
Left testis volume (mm ³)	285.2 + 30.0	0.5 + 0.3	Soma et al. (2003b)
Paired testes mass (g)	0.36 + 0.02	0.01 + 0.01	Tramontin & Brenowitz (1999)
Cloacal protuberance length (mm)	7.9 + 0.3	3.5 + 0.5	Soma et al. (2003b)
Plasma dehydroepiandrosterone (ng/ml)	0.80 + 0.11	0.73 + 0.09	Soma & Wingfield (2001)
Plasma androstenedione (ng/ml)	<0.1	< 0.1	Soma et al., unpublished results
Plasma testosterone (ng/ml)	4.18 + 0.79	0.16 + 0.01	Soma et al. (2003b)
Plasma estrone (ng/ml)	<0.1	<0.1	Soma et al., unpublished results
Plasma 17 β -estradiol (ng/ml)	<0.1–0.38	<0.1	Soma & Wingfield (2001)

Sedentary song sparrow populations (WA, BC).

*A five-level visual fat index (Helms & Drury, 1960) was used to score furcular and abdominal fat, which were then summed for each individual for analysis.

occurring adult neuroplasticity that is regulated by steroids (Tramontin & Brenowitz, 2000).

ESTROGEN EFFECTS ON SONG SPARROW BEHAVIOR AND BRAIN

Given that the testes are fully regressed and plasma sex steroid levels are basal in the nonbreeding season (Wingfield, 1994c; Soma & Wingfield, 2001; Soma et al., 2003), it was initially hypothesized that nonbreeding territorial aggression is not regulated by sex steroids in this species. Wingfield et al. made several additional observations in nonbreeding song sparrows in support of this idea, including: (1) plasma levels of testosterone do not rise in response to an STI during the nonbreeding season (Wingfield & Hahn, 1994); (2) removal of the regressed testes does not reduce aggression during an STI or impair the ability to maintain a nonbreeding territory (Wingfield, 1994b); (3) juvenile males with immature gonads successfully establish and defend territories in the nonbreeding season (Wingfield & Hahn, 1994; Nordby et al., 1999); and (4) increasing female sexual receptivity (via E_2 implants) does not affect sexual or aggressive behavior or plasma testosterone levels in nonbreeding males (Wingfield & Monk, 1994). Together, these data argued against a role for gonadal sex steroids in promoting nonbreeding aggression, especially because similar experimental manipulations in the breeding season do affect plasma testosterone levels and/or aggressive behavior (Runfeldt & Wingfield, 1985; Wingfield et al., 1989, 1990; Wingfield, 1994a).

Despite nondetectable concentrations of sex steroids in the general circulation during the nonbreeding season, the nonbreeding song sparrow brain expresses aromatase mRNA that is translated into active protein. Aromatase mRNA levels do not differ seasonally in brain areas strongly implicated in the regulation of aggression: nucleus taeniae (the avian homolog of the mammalian medial amygdala) and the ventromedial hypothalamus (Wacker et al., 2010). Also, aromatase activity in the ventromedial telencephalon (which contains nucleus taeniae) is high in both the breeding and nonbreeding season, but significantly reduced at molt, which parallels seasonal patterns of aggressive behavior (Soma et al., 2003). Furthermore, neither estrogen receptor alpha nor estrogen receptor beta mRNA levels differ across seasons in these brain regions (Wacker et al., 2010). Taken together, these studies raise the hypothesis that brain-generated estrogens are important for nonbreeding aggression, but the androgen substrate for brain aromatase might be of nongonadal origin during this season (Fig. 11–4).

The first study to investigate the role of sex steroids in nonbreeding territorial aggression, treated free-living male song sparrows with the steroidal aromatase inhibitor 1,4,6-Androstatrien-3,17-dione (ATD) in combination with the androgen receptor antagonist flutamide (via subcutaneous Silastic implants) (Soma et al., 1999). Seven days of ATD + flutamide treatment had no effect on territorial aggression. However, 30 days of ATD + flutamide treatment significantly decreased aggressive behaviors both during and after STI.

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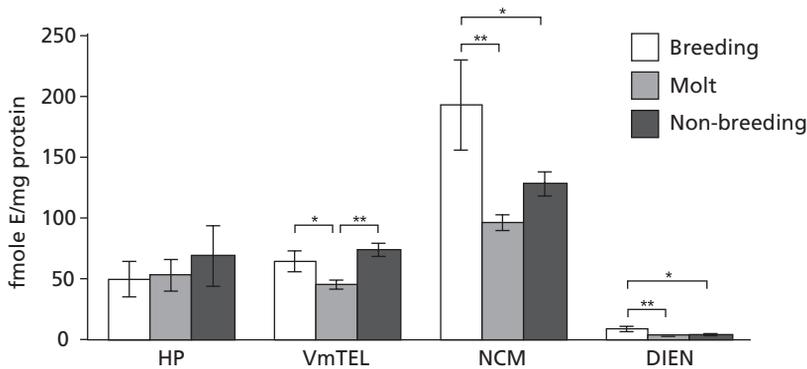


Figure 11–4. Seasonal Changes in Aromatase Activity in Wild Male Song Sparrow Brain. Free-ranging subjects were captured during the breeding season, molt, and the nonbreeding season. Aromatase activity was measured in hippocampus (HP), ventromedial telencephalon (vmTEL) including nucleus taeniae, caudomedial neostriatum (NCM), and diencephalon (DIEN). Brain tissue homogenates were incubated with tritiated androstenedione at 41°C, and the amounts of tritiated estrone and tritiated estradiol were measured. * $p < .05$, ** $p < .01$. Reprinted with permission from Soma, K. K., Schlinger, B. A., Wingfield, J. C., & Saldanha, C. J. (2003). Brain aromatase, 5 alpha-reductase, and 5 beta-reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior. *J Neurobiol*, 56(3), 209–221.

There were no effects of treatment on body mass or fat score, suggesting that treated subjects were healthy and foraging normally. When administered alone, flutamide has no significant effect on nonbreeding aggression in song sparrows (Sperry et al., 2010) or another songbird species, the European robin (Schwabl & Kriner, 1991). Taken together, these data suggest that nongonadal sex steroids, particularly estrogens, regulate nonbreeding territorial aggression in male song sparrows.

Importantly, the preceding study employed ATD as an aromatase inhibitor, which is not as potent as other aromatase inhibitors (Soma et al., 1999). To demonstrate more clearly a role for estrogens in nonbreeding aggression, a series of follow-up studies examined the behavioral effects of a more potent and specific nonsteroidal aromatase inhibitor, fadrozole hydrochloride (FAD) (Wade et al., 1994). Fadrozole was administered acutely or chronically to free-living male song sparrows in both the breeding and nonbreeding seasons (Soma et al., 2000a,b). For acute treatment, fadrozole was administered via intramuscular injection, and behavior was assessed 24 hours later. For chronic treatment, fadrozole was administered via subcutaneous miniosmotic pump, and behavior was assessed approximately 10 days later. In the nonbreeding season, both acute and chronic fadrozole treatments significantly reduced multiple measures of territorial aggression *during* an STI (Figs. 11–5 and 11–6) (Soma et al.,

and chronic effects of an aromatase inhibitor on territorial aggression in breeding and nonbreeding male song sparrows. *J Comp Physiol A*, 186(7–8), 759–769.

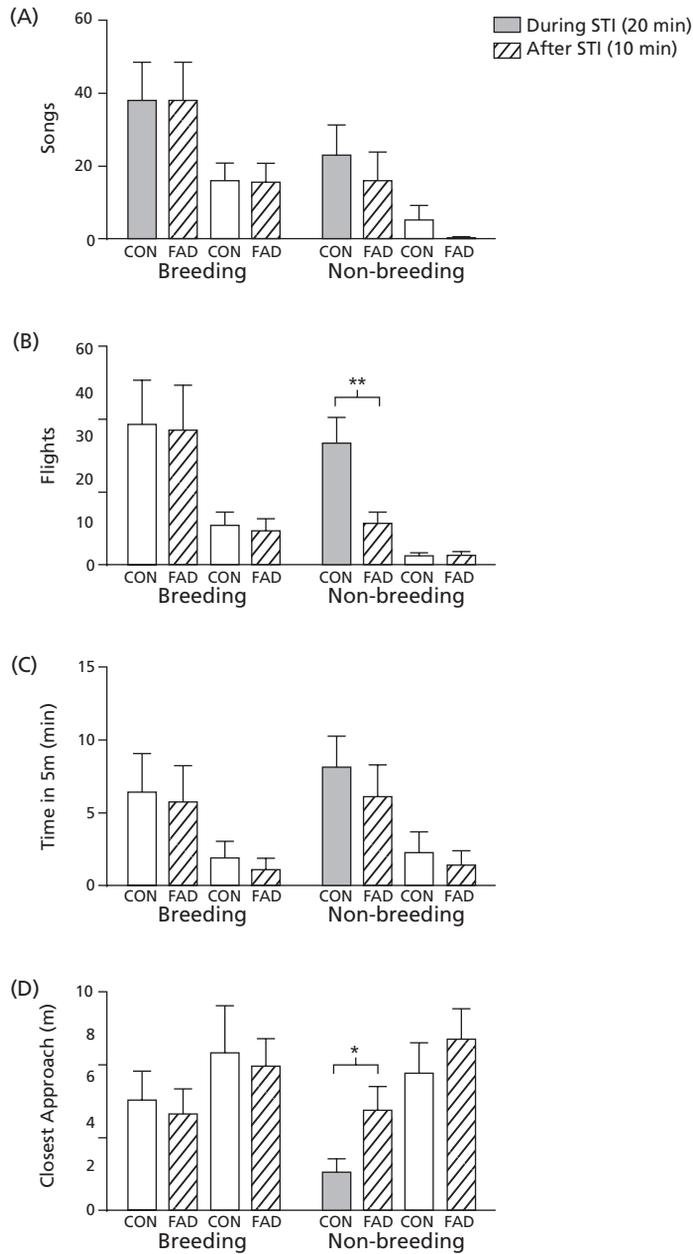


Figure 11-5. Acute Effects of the Aromatase Inhibitor Fadrozole or Vehicle on Territorial Behavior During (20 Minutes) and After (10 Minutes) a Simulated Territorial Intrusion. Subjects were wild adult male song sparrows during the breeding and non-breeding seasons. Subjects were tested 24 hours after FAD treatment (intramuscular injection). * $p < .05$, ** $p < .01$. Redrawn from data published in Soma, K. K., Sullivan, K. A., Tramontin, A. D., Saldanha, C. J., Schlinger, B. A., & Wingfield, J. C. (2000a). Acute

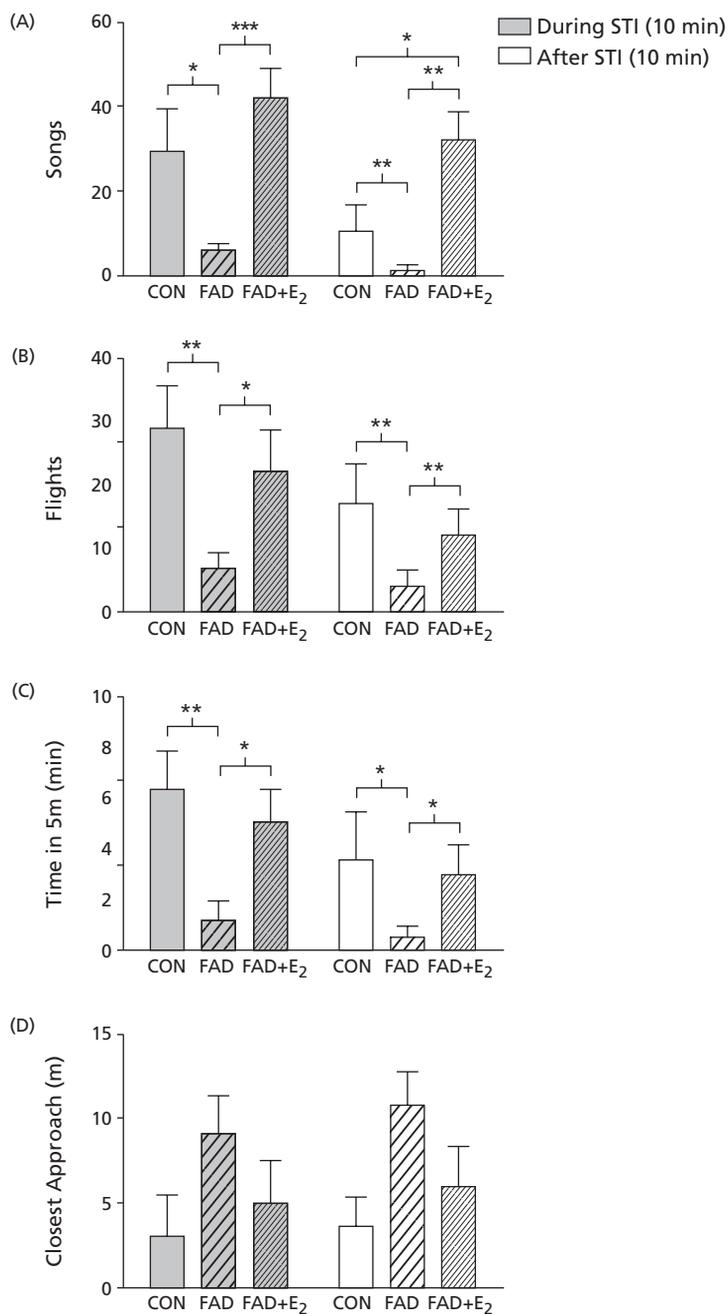


Figure 11-6. Chronic Effects of the Aromatase Inhibitor Fadrozole or Vehicle on Territorial Behavior During (10 Minutes) and After (10 Minutes) a Simulated Territorial Intrusion. Subjects were wild adult male song sparrows during the nonbreeding season.

2000a,b). Further, the effects of chronic fadrozole treatment on nonbreeding aggression were rescued by concurrent E₂ replacement (see Fig. 11–6) (Soma et al., 2000b). In contrast, neither acute nor chronic fadrozole treatment significantly affected territorial aggression in the breeding season (see Fig. 11–5) (Soma et al., 2000a). This lack of an effect of fadrozole on breeding aggression was likely owing to the fact that fadrozole causes a large increase in circulating testosterone levels in breeding subjects, which may have overcome the inhibition of aromatase activity and/or stimulated aggression via androgen receptors (Schlinger et al., 1999; Soma et al., 2000a). Fadrozole treatments did not significantly affect body mass or fat score, indicating that fadrozole did not reduce feeding behavior or debilitate subjects. Taken together, these studies show that estrogens are clearly involved in nonbreeding territorial aggression.

Interestingly, nonbreeding aggression *after* an STI was only affected by chronic fadrozole administration (Soma et al., 2000a,b) (see Figs. 11–5 and 11–6). Remarkably, E₂ replacement restored some features of post-STI aggression to control levels (flights and time in 5 meters), but elevated post-STI singing behavior above control levels to those typically seen during the breeding season (Soma et al., 2000a,b) (see Fig. 11–6). Taken together, these data suggest that persistence of territorial singing behavior after STI in the nonbreeding season may be tightly linked to long-lasting, genomic effects of E₂.

The effects of chronic fadrozole and E₂ treatments on the morphology of nuclei in the song control system were also investigated in nonbreeding song sparrows. Despite pronounced effects on territorial singing behavior, chronic fadrozole treatment did not significantly reduce HVC or RA volumes in nonbreeding song sparrows (Soma et al., 2004), perhaps because the song control system is already regressed at this time. In contrast, E₂ treatment significantly increased the volume of HVC to a size typical of the breeding season, indicating that E₂ can have profound neurotrophic and neuroanatomical effects during the nonbreeding season (Soma et al., 2004).

ANDROGEN SUBSTRATE FOR BRAIN AROMATASE IN THE NONBREEDING SEASON

These aromatase inhibitor studies clearly indicate that estrogens regulate nonbreeding territorial aggression as well as morphological aspects of behaviorally relevant brain regions. The androgen substrate for brain aromatase in the nonbreeding season was initially unclear, because circulating levels of testosterone and androstenedione are undetectable at this time (see Table 11–2). It was hypothesized that aromatizable androgens may be derived from the inactive

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Subjects were tested approximately 10 to 12 days after FAD treatment (subcutaneous Alzet pump). * $p < .05$, ** $p < .01$, *** $p < .001$. Redrawn from data published in Soma, K. K., Tramontin, A. D., & Wingfield, J. C. (2000b). Oestrogen regulates male aggression in the non-breeding season. *Proc Biol Sci*, 267(1448), 1089–1096.

prohormone dehydroepiandrosterone (DHEA). Dehydroepiandrosterone was measured in plasma from wild subjects using a specific radioimmunoassay, following dichloromethane extraction and Celite column chromatography (Soma & Wingfield, 2001). In the nonbreeding season, plasma levels of DHEA (0.8–1.75 ng/ml) were several times higher than plasma levels of testosterone and androstenedione. Moreover, the seasonal patterns of plasma DHEA levels and territorial behavior were similar: both are reduced during molt (Soma & Wingfield, 2001; Newman et al., 2008). The source of DHEA in the general circulation of nonbreeding birds may be either the regressed testes, the adrenals or the liver, which all have high DHEA levels (Soma & Wingfield, 2001; Newman & Soma, 2009, 2011). How DHEA levels in the general circulation are regulated remains unclear, as neither acute restraint stress nor GnRH administration affects DHEA levels in the brachial vein (Newman et al., 2008; Soma & Wingfield, 2001).

Next, wild nonbreeding male song sparrows were treated with DHEA to assess the effects on behavior and the brain (Soma et al., 2002). Importantly, this study used a dose of DHEA that elevated plasma DHEA levels within the physiological range. Chronic administration (>2 weeks) of DHEA (via subcutaneous Silastic implants) increased territorial singing behavior, including reduced song latency and increased persistence of singing after the STI. Surprisingly, other territorial behaviors (flights, closest approach, time in 5 meters) were not affected. Consistent with the effect on song, DHEA treatment also increased HVC volume by approximately 50% (to a size typical of the breeding season) (Soma et al., 2002; Newman et al., 2010). These effects of DHEA were similar to the chronic effects of E_2 on behavior (Soma et al., 2000b) and song system neuroanatomy (Soma et al., 2004) in the nonbreeding season. Thus, the behavioral and neuroanatomical effects of DHEA may occur through its conversion into active sex steroids, such as estradiol, within the brain.

Dehydroepiandrosterone cannot be directly aromatized by the aromatase enzyme and does not appear to bind to a classical intracellular steroid receptor with high affinity (Labrie et al., 2005). Rather, the enzyme 3β -hydroxysteroid dehydrogenase/isomerase (3β -HSD) converts DHEA into androstenedione, which can then be aromatized or converted by 17β -HSD into testosterone. 3β -HSD is abundant and active in the songbird brain, and 3β -HSD activity can be measured in vitro, using brain homogenates and tritiated DHEA as the substrate (Soma et al., 2004; Schlinger et al., 2008). Baseline 3β -HSD activity in brain areas implicated in song and aggression was assessed in wild male song sparrows across seasons. 3β -HSD activity was upregulated during the nonbreeding season in the central medial telencephalon (contains lateral septum and bed nucleus of the stria terminalis), ventromedial telencephalon (contains nucleus taeniae of the amygdala), and caudal telencephalon (contains RA) (Pradhan et al., 2010a). As mentioned, aromatase activity is also elevated in the ventromedial telencephalon during the nonbreeding season (Soma et al., 2003). Thus, the nonbreeding song sparrow brain can use

circulating DHEA to produce high local levels of aromatizable androgens and estrogens to activate territorial aggression, without increasing sex steroid levels in the general circulation (Schmidt et al., 2008; Pradhan et al., 2010b) (Fig. 11–7).

To gain further insight into the role of DHEA metabolism in the regulation of nonbreeding aggression, the effect of an STI on brain 3β -HSD activity was examined using *in vitro* enzyme activity assays to quantify the conversion of tritiated DHEA to its androgenic and estrogenic metabolites. Free-living male song sparrows were exposed to an STI or control (empty cage and silence) for 30 minutes. The STI rapidly increased 3β -HSD activity in the central medial telencephalon and caudal telencephalon in nonbreeding song sparrows (Pradhan et al., 2010b). Further, 3β -HSD activity in these regions was positively correlated with the amount of time the subject spent near the decoy (Pradhan et al., 2010b). These data suggest that the local production of aromatizable androgens increases in response to an aggressive encounter in the nonbreeding season. Importantly, the effect of STI on 3β -HSD activity was only observed when exogenous NAD^+ (cofactor for 3β -HSD) was not added to the enzyme activity assay (Pradhan et al., 2010b). When exogenous NAD^+ was added to the assay (cofactors are typically added at saturating levels to maximize the reaction rate), no effect of STI on 3β -HSD activity was detected. The role of cofactors

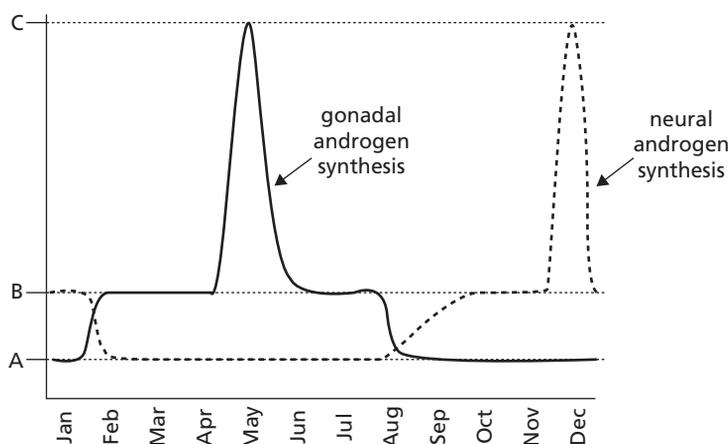


Figure 11–7. Hypothesized Patterns of Gonadal Androgen Synthesis (*Solid Line*) and Neural Androgen Synthesis (*Broken Line*) in Male Song Sparrows. Level A, constitutive levels of androgen synthesis for homeostatic functions; Level B, seasonal increase in androgen synthesis (regulated by predictable environmental cues); Level C, facultative and transient increase in androgen synthesis (regulated by unpredictable social cues). Reprinted with permission from Pradhan, D. S., Newman, A. E., Wacker, D. W., Wingfield, J. C., Schlinger, B. A., & Soma, K. K. (2010b). Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. *Horm Behav*, 57(4–5), 381–389.

in neurosteroid production is not well understood, but these data suggest that brain steroidogenic enzyme activity may be rapidly modulated by local bio-availability of cofactors. Alternatively, the social interaction might specifically affect 3 β -HSD in a subcellular compartment that is NAD-rich (e.g., mitochondria) (Pradhan et al., 2010a,b).

Surprisingly, in this study STI had no effect on the absolute amounts of formed tritiated estrogens in the central medial telencephalon and caudal telencephalon (Pradhan et al., 2010b). However, STI did decrease the amount of formed tritiated estrogens relative to total 3 β -HSD metabolites in these regions, indirectly suggesting that aromatase was inhibited by sustained aggressive behavior (Pradhan et al., 2010b). Research in Japanese quail has shown that dynamic social interactions lead to rapid and transient changes in brain aromatase activity. Specifically, brain aromatase activity in male quail is reduced after a 5-minute sexual interaction with a female, but is not significantly different from controls after 1- or 15-minute interactions (Cornil et al., 2005). Thus, in nonbreeding song sparrows, a reduction in brain aromatase activity after a 30-minute STI might reflect transient inhibition of aromatase activity. Given that sustained inhibition of aromatase activity inhibits nonbreeding aggression (Soma et al., 2000b), these data suggest that estrogenic regulation of aggressive behavior in the nonbreeding season may involve short pulses of local E₂ production. Future studies examining the effects of shorter STIs (e.g., 5 minutes) on 3 β -HSD and aromatase activities and on androstenedione, testosterone, and E₂ levels in the brain are needed to test this hypothesis.

LOCAL VERSUS SYSTEMIC PRODUCTION OF STEROIDS

Although neural metabolism of DHEA appears to be important for nonbreeding aggression in song sparrows, the source of DHEA substrate for brain 3 β -HSD remains enigmatic. During the nonbreeding season, levels of DHEA are high in the general circulation and are even higher in the regressed testes, adrenals and liver (Soma & Wingfield, 2001; Newman et al., 2008; Newman & Soma, 2009). Thus, DHEA may be secreted into the bloodstream by the gonads, adrenals, or liver and subsequently converted into active sex steroids in the brain (Labrie et al., 2005; Schlinger et al., 2008).

This hypothesis was tested by examining the effect of a 30-minute STI on circulating levels of DHEA. Simulated territorial intrusion had no effects on DHEA levels in the brachial plasma, gonads, or adrenals in the nonbreeding season (Soma & Wingfield, 2001; Newman & Soma, 2011) (Fig. 11–8). These data do not rule out the possibility of a peripheral source of DHEA substrate for brain 3 β -HSD. However, STI did elevate DHEA levels in the jugular plasma (Newman & Soma, 2011) (see Fig. 11–8), which in songbirds is enriched with brain-derived steroids (Schlinger & Arnold, 1992; Taves et al., 2011). Taken together, these data suggest that in addition to being synthesized

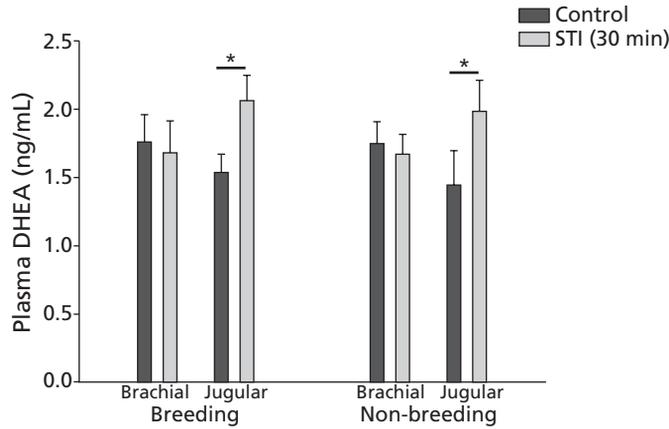
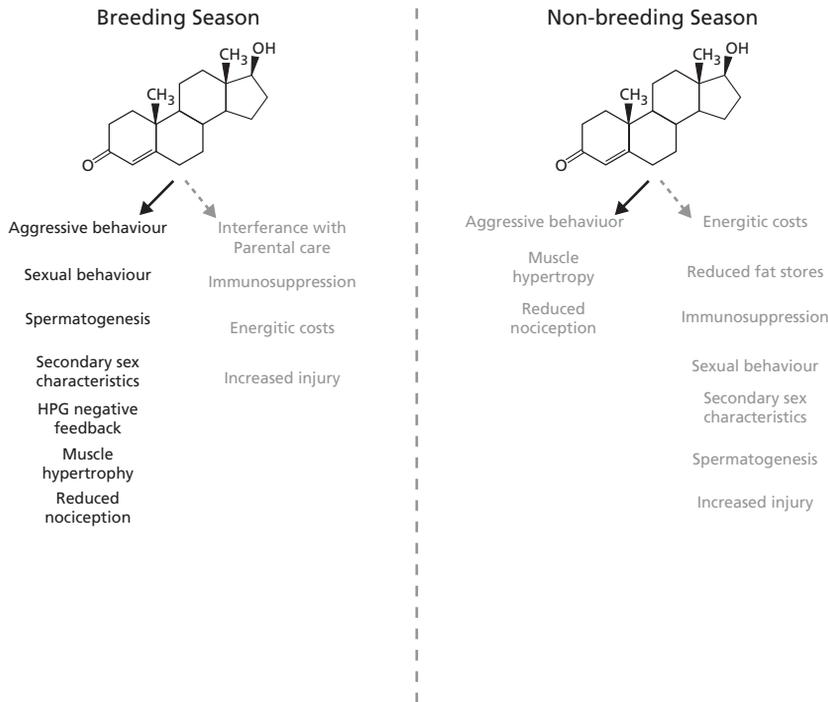


Figure 11–8. Effect of Simulated Territorial Intrusion for 30 Minutes on Brachial and Jugular Plasma Levels of Dehydroepiandrosterone During the Breeding Season and Nonbreeding Season $*p \leq .05$. Reprinted with permission from Newman, A. E., & Soma, K. K. (2011). Aggressive interactions differentially modulate local and systemic levels of corticosterone and DHEA in a wild songbird. *Horm Behav*, 60(4):389–396.

in the periphery, DHEA is synthesized *de novo* from cholesterol in the brain as a neurosteroid. Local production of DHEA is plausible given that the avian brain expresses the enzymes required for neurosteroid production, including cytochrome P450 side-chain cleavage (P450 scc) and cytochrome P450 17 α -hydroxylase/17,20-lyase (P450c17) (Tsutsui et al., 2006; Schlinger & Ramage-Healey, 2011).

Critically, these data suggest a shift from systemic to local sex steroid production and signaling in the brains of nonbreeding song sparrows (Soma, 2006; Schmidt et al., 2008) (Fig. 11–9). This shift may have evolved to avoid the costs of chronically elevated steroid hormones in the blood (Wingfield et al., 2001) (Fig. 11–10). For example, systemic testosterone treatment in the nonbreeding season suppresses cell-mediated and humoral immune responses in song sparrows (Owen-Ashley et al., 2004). Elevated circulating testosterone also decreases body mass and fat stores (Ketterson et al., 1991; Wikelski et al., 1999), which are essential for surviving snow storms and the low overnight temperatures during the nonbreeding season (see Fig. 11–1). Systemic testosterone also stimulates the development of secondary sexual characteristics (e.g., the cloacal protuberance) and activates reproductive behaviors, which are energetically expensive and inappropriate in the nonbreeding season. From an evolutionary perspective, the estrogenic regulation of male nonbreeding aggression may have become dissociated from systemic steroid signaling to reduce the exposure of peripheral tissues and other brain areas to testosterone and E₂. Importantly, this insight was made possible only by field studies of wild animals in their natural habitat.



Please note mention of color.

Figure 11–9. Actions and Costs of High Circulating Testosterone in the Breeding and Nonbreeding Season. Actions are in *black*, and potential costs are in *red*. In the breeding season, a major cost of high circulating testosterone is interference with parental care. In the nonbreeding season, interference with parental care is not an issue, but a major cost of high circulating testosterone is increased energy expenditure. Modified from Soma, K. K. (2006). Testosterone and aggression: Berthold, birds and beyond. *J Neuroendocrinol*, 18(7), 543–551.

CONCLUSIONS

Local production of steroids within the brain occurs across vertebrate species, including rodents and humans (Schlinger et al., 2001; Balthazart et al., 2006; Tsutsui et al., 2006; Taves et al., 2011). The data reviewed in this chapter suggest the intriguing hypothesis that local steroid synthesis may be especially important when circulating steroid levels are low, such as during the nonbreeding season, and perhaps also during early development, postmenopause, and various endocrine disorders. Thus, understanding the roles of locally synthesized steroids such as E₂ has important and broad implications for neuroendocrinology, in which the focus historically has been on steroids that are in the general circulation. We still know relatively little about how neurosteroids influence physiology and behavior, and a comparative approach will be critical

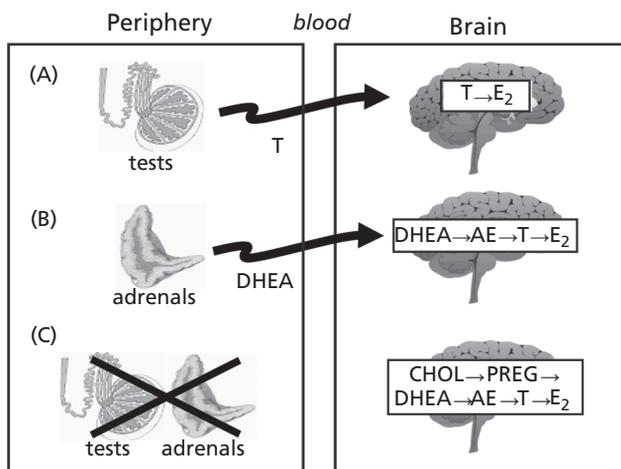


Figure 11–10. Potential Pathways for 17β-Estradiol Synthesis. A. Testosterone (T) can be synthesized in the gonads, released into the blood, travel to the brain, and be converted locally into E₂. B. The circulating prohormone dehydroepiandrosterone can be synthesized in the adrenals, released into the blood, travel to the brain, and be converted locally first into the aromatizable androgens T and androstenedione (AE) and then into E₂. C. 17β-Estradiol can be locally synthesized de novo from cholesterol in the brain. Modified from 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(3), 266–274.

for understanding the behavioral and functional consequences of neurosteroid production.

Future studies should further investigate neurosteroid production during times when circulating steroid levels are low. As one example, an important gap in our knowledge is the effect of STI on brain levels of testosterone, androstenedione, and E₂ and how these levels compare with plasma steroid levels. Local synthesis of steroids would be suggested if steroid levels were higher in brain tissue than in the circulation after STI. As a second example, the effects of STI on hypothalamic gene expression in the breeding and nonbreeding season were recently characterized by microarray (Mukai et al., 2009), but future research should also examine the effects of E₂ on gene expression in the songbird brain. Such studies have been greatly facilitated by the recent sequencing of the zebra finch genome and microarrays designed for use with songbird brain tissue (Replogle et al., 2008). Finally, it has been hypothesized that local steroid signals are more likely than systemic steroid signals to act via rapid, nongenomic mechanisms (Woolley, 2007; Schmidt et al., 2008; Heimovics et al., 2012). Thus, another important avenue of future research will be to explore the cellular and molecular mechanisms mediating the effects of E₂ on nonbreeding aggression.

AQ2: Please provide complete details of "Heimovics, et al., 2012; Tramontin and Brenowitz (1999)" in the reference list.

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