8 Novel Mechanisms Underlying Neuroendocrine Regulation of Aggression: A Synthesis of Rodent, Avian, and Primate Studies

G. E. Demas · M. A. Cooper · H. E. Albers · K. K. Soma

Abstract: An import role for gonadal steroid hormones, particularly testosterone, in mediating aggressive behavior is well-established across vertebrate taxa. Due to the emphasis placed on testosterone much less is know regarding the potential role of other steroid and peptide hormones in the regulation of aggression. The idea that any single hormone mediates aggression, however, is overly simplistic. In fact, research over the last two decades or more have has suggested that aggression in a complex behavior that is regulated by a wide range of hormones in addition to testosterone. This chapter reviews several novel neuroendocrine mechanisms that have been recently identified as being implicated in the regulation of aggressive behavior. Specific focus is placed on studies conducted in rodents, birds and primates, as the majority of research in this area has focused on these groups of animals.

List of Abbreviations: ACTH, adrenocorticotropic hormone; ADHD, attention deficit hyperactivity disorder; AE, androstenedione; ALLO, allopregnanolone; AR, androgen receptor; Aromatase, cytochrome P450 aromatase; AVP, arginine vasopressin; AVT, arginine vasotocin; BERKO, estrogen receptor β knock-out; BNST, bed nucleus of the stria terminalis; CNS, central nervous system; CORT, cortisol; 5α-DHT, 5α-dihydrotestosterone; DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; E_1, estrone; E_2, 17β-estradiol; ER, estrogen receptor; ERKO, estrogen receptor knock-out; GABA, gamma-aminobutyric acid; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase/isomerase; HP, hippocampus; HPA, hypothalamo-pituitary-adrenal; LD, light:dark; LH, luteinizing hormone; MPOA, medial preoptic area; N, nidopallium; NCM, caudomedial nidopallium; P450c17, cytochrome P450 17α-hydroxylase/C17,20 lyase; P450scc, cytochrome P450 side chain cleavage; POA, preoptic areaPREG, pregnenolone; PROG, progesterone; RA, robust nucleus of the arcopallium; STI, simulated territorial intrusion; T, testosterone; Tn, nucleus taeniae of the amygdala; TP, testosterone propionate; VMN, ventromedial nucleus

1 Introduction

► "It is the habit of every aggressor to claim that it is acting on the defensive" (Jawaharlal Nehru)

Among the wide array of social behaviors displayed by organisms, undoubtedly one of the most important and well studied is aggression. Aggression is a highly complex behavior displayed by virtually all living organisms, and serves a wide range of adaptive functions. The possibility for aggressive behavior exists whenever the interests of two or more individuals are in conflict, typically involving limited resources (e.g., food, territories, and mates). In nature, social interactions dictate which animals gain access to a limited resource and which ones do not. In many cases, a submissive posture displayed by one animal avoids the necessity of physical aggression. Additionally, animals may engage in threat displays or ritualized combat in which dominance is determined in the absence of physical harm. If such displays are ineffective, however, physical aggression can result. In some cases a material goal for aggression cannot be identified, and animals appear to fight over dominance status (cf. Mason, 1993). Aggression is an essential part of the socialization process, as mothers and other adults use aggression to modify inappropriate behavior in developing animals.

Despite its importance, aggression is a notoriously nebulous concept that has been defined and categorized in a multitude of ways over the years. Aggression has traditionally been defined as overt behavior with the intention of inflicting physical damage upon another individual or "goal entity" (Moyer, 1971). One of the most commonly employed classifications of aggression was described by Moyer (1971), who divided aggression into specific subtypes based on differences in social conditions in which the behavior was elicited. Among these forms of aggression were predatory aggression, intermale aggression, fear-induced aggression, irritable aggression, maternal aggression, territorial defense, and instrumental aggression. One of the primary tenets of Moyer's classification system was that, although these different forms of aggression share behavioral features, the environmental factors eliciting these responses and the biological substrates underlying their manifestation differ markedly. More recently, a simplified classification of aggression has been suggested (Blanchard and Blanchard, 1988) in which

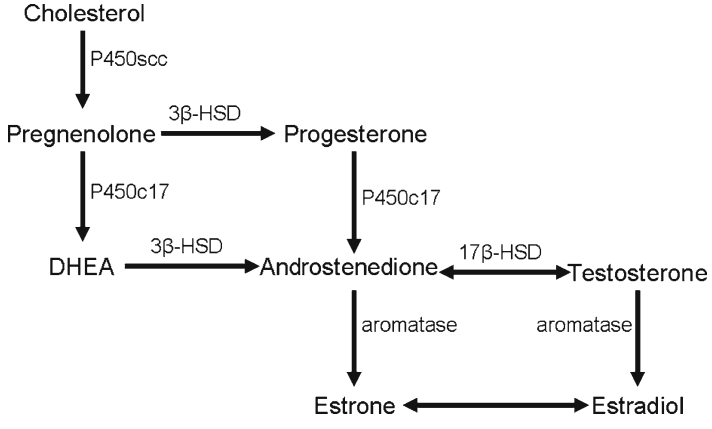
aggression is divided into *offensive* and *defensive* aggression. Offensive aggression refers to behaviors used in attack, whereas defensive behaviors do not involve an active approach to the opponent; rather, they serve as a defense against an attack. This latter classification system provides a useful framework with which to identify and describe aggressive behavior across many species.

Aggressive behavior has received extensive study under a wide range of environmental settings and experimental conditions; the experimental models used, the types of aggression measured, and the species tested, however, can vary considerably from study to study. Thus, it is often difficult to compare results across a range of studies. Although a relatively large number of experimental paradigms have been developed to test aggression (e.g., electric shock or brain lesion-induced aggression, conditioned aggression), one of the most prevalent models of assessing offensive aggression in rodents has been the resident–intruder model. This test is intended to simulate rodent territorial aggression and involves introducing a group-housed, nonexperimental "intruder" (typically younger and smaller than the experimental animal) into the home cage of an experimental animal, and the amount and duration of aggressive behavior (e.g., chases, attacks, bites) are subsequently recorded in a timed test. Another less-commonly employed but useful model is aggression in a neutral arena, which involves placing two animals in a novel "neutral" cage and recording the amount of aggression directed toward each animal. In addition, this latter model has the added benefit of assessing the formation of dominance relationships among animals because "territories" have not been established at the time of testing. Finally, it is important to consider the time of testing (i.e., day versus night), as most rodents are nocturnal and display significantly more behavior during night; many primates in contrast are diurnal. Thus, behavioral testing is more appropriately assessed during the day.

Much of the early research on the neuroendocrine mechanisms of aggression focused on the role of gonadal steroid hormones, and predominantly testosterone (T), as the primary factor regulating aggression (*Figure 8-1*). In fact, most behaviorally oriented textbooks discuss the role of T in aggression almost exclusively, with much less emphasis placed on the role of other factors (e.g., neuropeptides, neurotransmitters) in mediating this behavior. One of the goals of this chapter is to suggest that the idea that a single hormone or neurotransmitter mediates aggression is overly simplistic. For example, recent evidence indicates that T can be converted to 17β-estradiol within the brain and that estrogens may mediate aggressive behavior, at least in some species and contexts (Soma et al., 2000a, b). Alternatively, the so-called

Figure 8-1

Simplified diagram of sex steroid synthesis. *Steroids*: PREG = pregnenolone; PROG = progesterone; DHEA = dehydroepiandrosterone; AE = androstenedione; T = testosterone; E_1 = estrone; E_2 = 17β-estradiol. *Enzymes*: *P450scc* = Cytochrome P450 side chain cleavage; *P450c17* = Cytochrome P450 17α-hydroxylase/C17,20 lyase; *3β-HSD* = 3β-hydroxysteroid dehydrogenase/isomerase; *17β-HSD* = 17β-hydroxysteroid dehydrogenase; *Aromatase* = Cytochrome P450 aromatase



"weak" androgens such as dehydroepiandrosterone (DHEA) may be produced in extragonadal tissue (e.g., adrenals) or *de novo* within the brain. In fact, it has been suggested that both adrenal and neurally derived androgens, the latter called neurosteroids (Baulieu, 1991), may play an important role in regulating aggressive responses (Simon, 2002). Collectively, these important findings have helped elucidate several possible pathways by which androgens can act on the brain, either directly or indirectly, to affect aggression (*Figure 8-2*).

In addition to androgens, a wide range of neuroendocrine factors have now been established as playing a major role in aggressive behaviors. We will review important classical findings, as well as more recent discoveries for some of the key factors; however, an encyclopedic review of all the neuroendocrine factors regulating aggression is beyond the scope of this chapter. For a more in-depth discussion of specific neuroendocrine factors and their effects on aggression, we refer the reader to some excellent recent reviews (Ferris and Delville, 1994; Albers and Bamshad, 1998; Ferguson et al., 2002; Chiavegatto and Nelson, 2003).

Another important goal of this chapter is to present a comparative approach to the study of aggression. Historically, much of the early work on the physiology of aggression placed considerable emphasis on rodent models (e.g., Edwards et al., 1969; Brain and Poole, 1974). In fact, much of this research has focused on highly domesticated species (e.g., inbred strains of rats and mice). Although these models have provided important insights into the mechanisms of aggression, and continue to do so (especially in the light of recent advances in molecular and genetic techniques), recent evidence suggests that specific mechanisms mediating aggression can differ markedly across taxa (reviewed in Wingfield et al., 1997). Thus, an important aim of this chapter is to integrate research findings from several different taxa with the goal of elucidating common themes and noteworthy differences, with respect to the neuroendocrine regulation of aggression. With this goal in mind, we have chosen to focus on three groups of animals – rodents, birds, and primates—where considerable data from both laboratory and field settings are available. The application of a comparative approach to the study of aggression will allow for the development of a more comprehensive understanding of the factors mediating social behavior.

2 Neuroendocrine Regulation of Aggression in Rodents

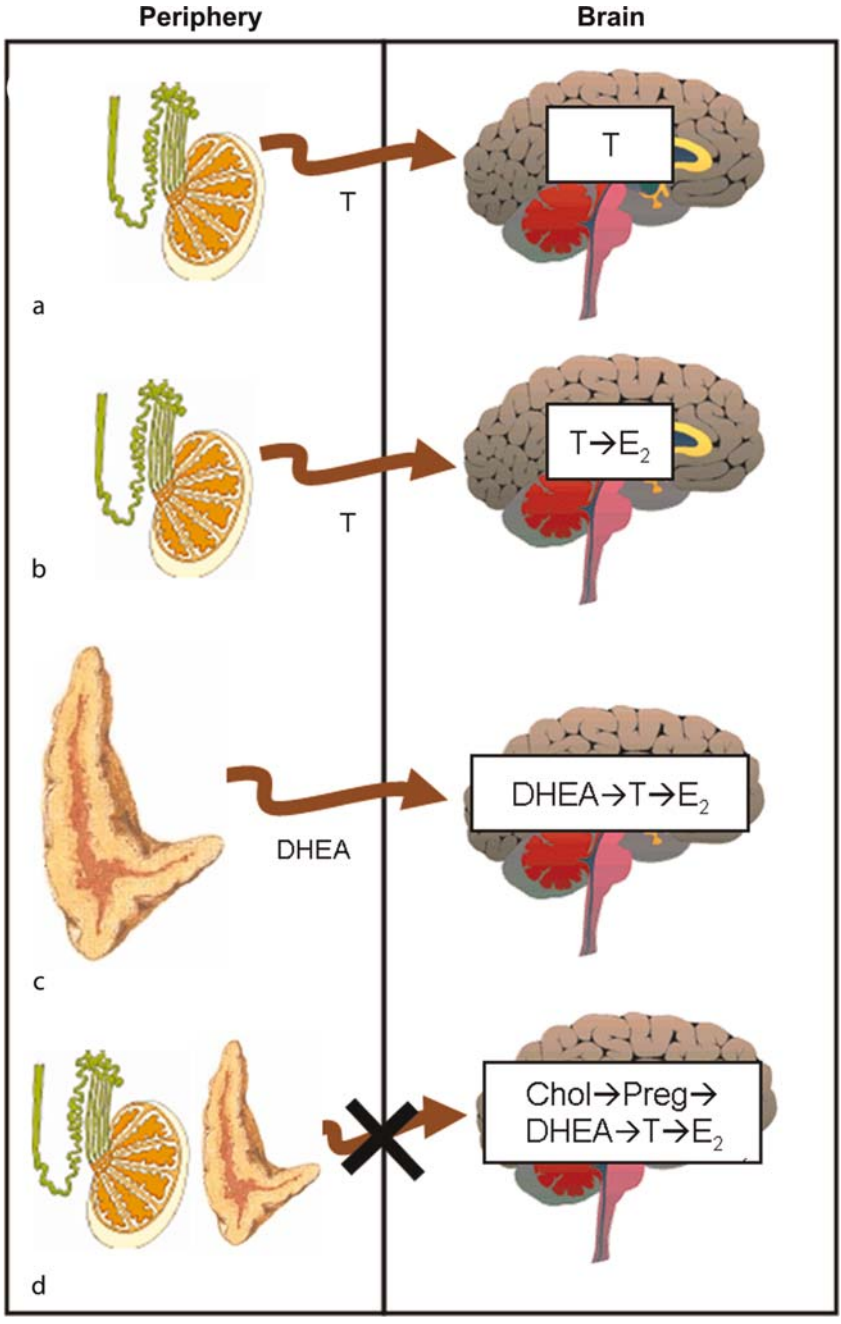
Much of the early work on the neuroendocrine regulation of aggression focused on the role of gonadal steroids in mediating intermale aggression and a majority of this work was conducted using laboratory-bred strains of rats and mice. Although space constraints do not permit an exhaustive discussion of this literature, a review of the major findings in this area will serve as a foundation for interpreting more recent findings, as well as exploring alternative physiological mechanisms regulating aggression in rodents.

2.1 Androgens: Organizational Versus Activational Effects

The link between T and aggression in rodents was first reported by Uhrich, who demonstrated that castration of postpubertal male rats decreased aggression, and that T replacement substantially reversed this effect (Uhrich, 1938). Since this initial finding, literally thousands of papers have been published examining the relationship between androgens and aggression (Beeman, 1947; Bevan et al., 1958, 1960; Yen et al., 1962; Sigg et al., 1969; Suchowsky et al., 1969; Brain and Haug, 1992). An important distinction must be made, however, between *organizational* and *activational* effects of androgens on aggression. Ever since the initial findings of Young and colleagues studying the effects of steroids on reproductive behaviors in guinea pigs, a well-established tenet of behavioral endocrinology is that steroid hormones can exert differential effects on physiology and behavior, depending on the time course of exposure during development (Phoenix et al., 1959). For example, for some behavioral responses (e.g., reproduction), androgens are required during a critical or "sensitive" period of perinatal development to permanently organize the neuroendocrine substrates required for adequate display of appropriate responses during adulthood. In many cases, the presence of androgens during adulthood is also required to activate the appropriate responses.

Figure 8-2

Steroid hormones can reach the brain to affect aggression via several possible mechanisms. (a) Gonadal testosterone can act directly on the brain; (b) gonadal T can be converted locally to estradiol; (c) adrenal DHEA can be converted locally to T or E_2; (d) neurosteroids can be produced locally in the absence of gonadal and adrenal steroid production



A large number of studies have demonstrated both organizational and activational effects of androgens on aggression in rodents. For example, castration of male mice on the day of birth eliminates aggression during adulthood, despite the presence or absence of exogenous androgens at the time of testing (Bronson and Desjardins, 1969). However, treatment of castrated pups with exogenous T immediately following surgery will result in normal levels of male aggression in adult animals, provided that androgens are given at the time of behavioral testing. Interestingly, the level of aggression displayed by adult female mice can be increased to that seen in male mice by treatment with exogenous androgens early during development, again provided that injections of hormone are also administered at the time of testing, consistent with an organizational effect of androgens on aggression (Edwards, 1968; Bronson and Desjardins, 1970). Male-like aggression in females treated with T prepubertally is not seen if T is not administered in adulthood, suggesting that activation of aggression is androgen-dependent.

The sensitive period for the organizational effects of androgens appears to be relatively long, although exogenous treatment with T is most effective when administered close to the time of birth in mice. For example, a single injection of T in castrated mice is sufficient to organize adult aggression, but this treatment is more effective on Day 0 than on Day 10 after birth (Edwards, 1969; Bronson and Desjardins, 1970). Furthermore, single injections of T become ineffective between 12–24 days of postnatal age, although more prolonged treatment (e.g., 20 days) can effectively organize adult aggression as late as 30 days postnatal (Edwards, 1970).

The widely accepted relationship between the presence of the testes (and normal circulating T concentrations) and aggression, however, is based primarily on studies of male–male aggression in a limited number of highly domesticated species such as laboratory rats (*Rattus norvegicus*) and house mice (*Mus musculus*) typically maintained in 12:12 light—dark (LD) cycles and tested during the light phase. In these species, removal of the gonads results in substantial decreases in circulating T and subsequently, reduced aggression (e.g., Edwards, 1969, 1970). When males of several nondomesticated species are examined, however, exceptions to the relationship of low circulating T concentrations and reduced aggression begin to emerge (Mathewson, 1961; Tiefer, 1970; Whitsett, 1975; Caldwell et al., 1984; Demas et al., 1999; Wiley and Goldizen, 2003; Gottreich et al., 2001). For example, several species of animals, including Mongolian gerbils (*Meriones unguiculatus*), prairie voles (*Microtus ochrogaster*), Syrian hamsters (*Mesocricetus auratus*), blind mole rats (*Spalax Ehrenberg*), saddle-back tamarins (*Saguinus fuscicollis*), European starlings (*Sturnus vulgaris*), and red-sided garter snakes (*Thamnophis sirtalis*) do not display decreased aggression after castration (Davis, 1957; Mathewson, 1961; Tiefer, 1970; Christianson et al., 1972; Epple, 1978; Dewsbury, 1991; Demas et al., 1999b). However, even in laboratory species, T does not stimulate aggression under all conditions. For example, in rats, T appears to be most effective in stimulating aggression during competitive experiences, but not during other social encounters (Albert et al., 1992).

2.2 Androgens and Seasonal Aggression

Several studies have examined the role of T in aggression *indirectly* by manipulation of the photoperiod. Many nontropical rodent species are seasonal breeders, maintaining reproductive function during summer and curtailing breeding during the winter. Ambient day length (photoperiod) is the proximal environmental cue used by individuals within these species to coordinate their reproduction to the appropriate season (Goldman, 2001). For example, reproductive function (and high levels of circulating T) is maintained during long "summer-like" days (e.g., >12.5 h of light/day) whereas reproductive regression, including virtual collapse of the gonads and marked decreases in T occur during the short "winter-like" days (i.e., <12.5 h of light/day) (Goldman, 2001). Interestingly, in male Syrian hamsters maintaining animals in short days actually *increases* resident–intruder aggression compared with long-day hamsters (Garrett and Campbell, 1980). Specifically, adult male Syrian hamsters housed in short days for nine weeks display approximately twice the amount of aggression in a resident–intruder test compared with long-day controls when tested 4 h before dark, despite gonadal regression (Garrett and Campbell, 1980). After prolonged maintenance in short days (>15 weeks) hamsters typically undergo spontaneous gonadal recrudescence

(i.e., increased testicular mass and circulating T), despite continued maintenance in short days. The short-day increases in aggressive behavior largely disappear in animals undergoing spontaneous recrudescence, returning to long-day levels of aggression by 21 weeks (Garrett and Campbell, 1980). More recently, short-day increases in aggression in male Syrian hamsters have been confirmed (Jasnow et al., 2002; Caldwell and Albers, 2004). For example, Syrian hamsters housed in short days (LD 10:14) for 10 weeks displayed a significantly greater number of attacks and a longer duration of attacks than did long-day hamsters when tested using a resident–intruder test (Jasnow et al., 2002). Furthermore, timed daily melatonin injections mimicking short-day patterns of the hormone in long-day, pineal-intact animals will produce short-day-like increases in aggression. Because these injections occurred for only 10 days, the gonadal mass and circulating levels of T are unaffected, supporting the idea that photoperiodic changes in aggression are not mediated by changes in gonadal steroids in this species (Jasnow et al., 2002). In contrast, these results suggest that levels of aggressive behavior are mediated by changes in the pattern of melatonin secretion.

Photoperiodic changes in aggression have been demonstrated in females of at least one species, Syrian hamsters (Fleming et al., 1988; Badura and Nunez, 1989). Female hamsters were housed in long (LD 14:10) or short days (LD 6:18) for 12 weeks and then both offensive and defensive aggression were tested (Fleming et al., 1988). Female hamsters maintained in short days displayed significantly less defensive aggression compared to long-day animals and thus had a higher ratio of offensive to defensive aggression than long-day animals (Fleming et al., 1988).

In virtually all mammals, photoperiodic responses are mediated by changes in the pineal indolamine melatonin. Melatonin is secreted in abundance during darkness, whereas daylight inhibits pineal melatonin secretion (Goldman, 2001). Thus, changes in ambient day length result in changes in the pattern of secretion of melatonin. In this manner, it is the precise pattern of melatonin secretion, and not the amount of hormone per se, that provides the biochemical "code" for day length (Goldman, 2001).

Pinealectomy, which eliminates melatonin secretion and renders animals physiologically "blind" to day length, prevents the short-day increase in aggression in female Syrian hamsters, whereas treatment of long-day hamsters with exogenous short-day-like melatonin increases aggression in female Syrian hamsters (Fleming et al., 1988). Ovariectomy, in contrast, has no effect of aggression. This finding suggests that photoperiodic changes in aggression are independent of changes in gonadal steroids in female Syrian hamsters (Fleming et al., 1988). A subsequent study in female Syrian hamsters confirmed these findings and provided further support for a role of pineal melatonin in mediating photoperiod changes in aggression. Specifically, a higher percentage of female hamsters housed in short days (LD 6:18) showed aggressive behavior compared with long day housed (LD 16:8) hamsters (Badura and Nunez, 1989). Consistent with previous findings, short-day aggression was attenuated by pinealectomy, but treatment with exogenous estradiol (E_2) (alone or in combination with progesterone) had no effect on aggression. These results support the hypothesis that photoperiodic changes in aggression are mediated by pineal melatonin, but independent of gonadal steroids, at least in female Syrian hamsters.

In Syrian hamsters, unlike most rodent species, females are more aggressive than males (Marques and Valenstein, 1977; Ciacco et al., 1979). Few studies have examined the role of photoperiod on male aggression in rodents displaying typical male-dominant aggression. Unlike Syrian hamsters, male Siberian hamsters (*Phodopus sungorus*) display significantly more aggression than females. It has recently been demonstrated that short-day male Siberian hamsters are significantly more aggressive than long-day animals (Jasnow et al., 2000; Jasnow et al., 2002), consistent with previous studies in Syrian hamsters. Specifically, male Siberian hamsters housed in short days (LD 8:16) for 10 weeks display a greater number of attacks during a resident–intruder test and have a lower latency to initial attack, relative to long-day (LD 16:8) animals. As previously reported for many rodent species, prolonged maintenance on short days (i.e., 20 weeks) resulted in spontaneous reproductive recrudescence in which the gonads, and thus T, returned to normal long-day levels (Jasnow et al., 2000). Interestingly, gonadally recrudesced hamsters displayed less aggression than gonadally regressed animals even though both groups experienced the same photoperiod and melatonin signal; levels of aggression in recrudesced hamsters were generally indistinguishable from long-day hamsters (Jasnow et al., 2000). These results support previous findings in male Syrian hamsters (Garrett and Campbell, 1980). When short-day Siberian hamsters were implanted with Silastic capsules

containing T (to achieve long-day-like levels), aggression actually *decreased* compared with short-day control animals (Jasnow et al., 2000), suggesting that short-day increases in aggression may be *inversely* related to serum T concentrations.

Despite growing evidence that short-day increases in aggression are independent of (or inversely related to) circulating levels of T, much less is known about the precise neuroendocrine mechanisms underlying seasonal aggression in rodents. As previously described, several studies have implicated changes in the pineal hormone melatonin in mediating short-day aggression. More recent research in male Siberian hamsters (Demas et al., 2004) confirms previous findings that treatment of long-day animals with short-day-like levels of melatonin mimics photoperiodic changes in aggression; long-day hamsters given daily timed injections of melatonin 2 h before lights-out to mimic short-day levels of the hormone displayed elevated aggression in a resident–intruder test compared with control animals. As with previous studies, these results were not likely due to changes in gonadal steroids, as serum T was unaffected by this injection protocol.

The effects of melatonin on aggression in rodents may be due to direct actions of this hormone on neural substrates mediating aggression (e.g., hypothalamus, limbic system). Alternatively, melatonin-induced aggression may be indirectly due to changes in hypothalamo–pituitary–adrenal (HPA) activity, as adrenal hormones have been implicated in aggressive behavior (Haller and Kruk, 2003). In support of the latter hypothesis, changes in both the size and function of the adrenal gland are associated with changes in aggression (Paterson and Vickers, 1981). In addition, male house mice housed in a LD 12:12 photoperiod and treated with melatonin display increased territorial aggression but decreased adrenal masses compared to saline-treated animals (Paterson and Vickers, 1981). The increases in aggression displayed by melatonin-treated animals, however, can be blocked by adrenalectomy (Paterson and Vickers, 1981). Experimental reductions in both adrenomedullary catecholamines, as well as adrenocortical glucocorticoids, are associated with decreased aggression in rodents (Patterson and Vickers, 1981; Haller and Kruk, 2003) and reductions in glucocorticoids via pharmacological blockade of adrenocorticotropic hormone (ACTH) release can attenuate melatonin-induced increases in aggression in mice (Patterson and Vickers, 1981). Thus, exogenous melatonin, despite reducing adrenal mass, appears to increase aggression by stimulating adrenocortical steroid release. These results are particularly intriguing given that house mice have traditionally been assumed to be photoperiodically nonresponsive (Nelson, 1990).

More recently, research has implicated changes in adrenocortical hormones in mediating melatonin- and possibly short-day-induced aggression in Siberian hamsters. As described previously, long-day hamsters treated with short-day-like levels of melatonin displayed increased aggression, comparable to levels seen in short-day animals (Demas et al., 2004). Interestingly, melatonin-induced aggression could be blocked by bilateral adrenalectomy, consistent with previous results in house mice (Paterson and Vickers, 1981). Adrenal demedullation, which eliminates adrenal catechloamines (i.e., epinephrine) but leaves adrenocortical steroid release (i.e., cortisol, DHEA) intact, had no effect on melatonin-induced aggression (Demas et al., 2004). Collectively, these results support the hypothesis that the effects of exogenous melatonin on aggression are mediated by the effects of this hormone on adrenocortical steroids. However, it is currently not known which class of steroid hormones may mediate this effect, as adrenal androgens (e.g., DHEA) and glucocorticoids (e.g., cortisol) have both been implicated in aggression in rodents (Schlegel et al., 1985; Haller and Kruk, 2003). In laboratory rats and mice, corticosterone (CORT) is the predominant adrenal glucocorticoid, and these species secrete little to no adrenal DHEA. In contrast, in hamsters, as in humans, cortisol is the primary adrenal glucocorticoid, and both hamsters and humans secrete measurable amounts of DHEA and its sulfated form, DHEA-S (Pieper et al., 2000; Mellon and Vaudry, 2001).

Recent evidence in avian species suggests that aggression in the nonbreeding season (i.e., winter) may be mediated by changes in DHEA (Soma et al., 2000; see Section 4). Although similar evidence suggesting a role for DHEA in mediating photoperiodic changes in aggression in rodents is lacking, studies in mice suggest that exogenous melatonin can stimulate DHEA production from cultured adrenal glands (Haus et al., 1996). Behavior was not examined in this study; however, these results are consistent with the hypothesis that short-day increases in melatonin may increase adrenal production of DHEA and thus affect aggression.

Several field studies published to date support the laboratory data discussed above suggesting differential dependence on gonadal steroids in animals during breeding season versus when they are not breeding. Specifically, male rat-like hamsters (*Cricetus triton*) in the field display elevated aggression during the winter nonbreeding season, despite low levels of plasma T (Zhang et al., 2001). Seasonal changes in aggression appear independent of seasonal changes in circulating T in wild wood rats (*Neotoma fuscipes*) (Caldwell et al., 1984). Pronounced seasonal changes in aggression are seen in male wood rats, with high levels during midbreeding season and low levels during the nonbreeding season. Despite differences in circulating T levels at these two time points, castration has no affect on aggression, suggesting an independence of seasonal aggression from circulating levels of T (Caldwell et al., 1984). More recently, seasonal changes in aggressive encounters have been examined in free-living arctic ground squirrels (Buck and Barnes, 2003). The effects of challenges by conspecific males on circulating T levels varied seasonally, with challenges by male intruders eliciting significant increases in circulating T during the spring breeding season, but similar challenges failed to trigger increase in androgen at the end of the summer after the breeding season. These results suggest that androgens play a more important role during the breeding season than during the nonbreeding season (i.e., late summer). Collectively, these studies fail to support the simple notion that all forms of aggression are mediated by circulating T by providing salient examples of T-*independent* aggression, at least with respect to circulating levels of the hormone. Unlike other forms of aggression, however, very little is known regarding the neuroendocrine mechanisms underlying seasonal changes in aggression in mammals.

2.3 Estrogens and Aggression

The specific role of estrogens in the regulation of aggression is complicated by the fact that both estrogens and androgens (if aromatized to estrogens) can act on estrogen receptors (ER) within the brain. Thus, many of the effects of androgens on aggression traditionally ascribed to actions via androgen receptors (AR) may in fact be due to activation of ER following aromatization. In addition, unlike the AR, two isoforms of ER, ERα, and ERβ, have been identified, and these receptors can exert differential effects on rodent aggression (Nomura et al., 2002). Much of the experimental evidence implicating ER in the regulation of intermale aggression has been determined using genetic knockout (KO) techniques in inbred male mice. For example, initial studies using ERKO mice that lack functional ERα demonstrated that intermale aggression was markedly reduced and male-typical offensive attacks were rarely displayed by ERKO mice (Ogawa et al., 1997). Moreover, both castrated wild-type (WT) and ERKO mice displayed comparably low levels of aggression; T replacement increased aggression in castrated WT males, but had no effect on ERKO aggression (Ogawa et al, 1998).

Unlike studies using ERKO mice, male mice that lack functional ERβ (BERKO) display relatively normal or even slightly elevated levels of aggressive behavior compared with WT males (Ogawa et al., 1999). Male aromatase KO mice that lack a functional aromatase enzyme necessary for the conversion of androgens to estrogens also display marked reductions in aggression (Matsumoto et al., 2003). This finding, coupled with the data from ERKO studies, suggests that the effects of androgens on intermale aggression may be due, at least in part, to conversion of these hormones to estrogens and the subsequent action of estrogens on ER within the brain. Furthermore, these results support the idea that ERα is the active form of ER for regulating neuroendocrine effects on aggression (Ogawa, 2003), although more research is needed. These results suggest that ERβ may provide a "brake" on aggression.

2.4 Glucocorticoids and the Development of Aggression

Although many pre-pubertal rodents display some form of "play aggression" prior to the development of fully functioning testes (Delville et al., 2003), fighting among males typically begins at the onset of puberty. Postpubertal aggression is mediated by the surge in circulating steroids that can "activate" aggressive behavior. Much of the work in this area has been performed in Syrian hamsters. For example, repeated

social stress in male Syrian hamsters inhibits aggression and increases submissive behaviors in adulthood. Acute social defeat in adult male Syrian hamsters increases subsequent submissive behavior and produces a complete loss of normal territorial aggression (Huhman et al., 2003). This change in agonistic behavior, however, appears mediated by the CNS and not by glucocorticoid feedback (Cooper and Huhman, 2003). Interestingly, recent evidence suggests that social stress during puberty enhances offensive aggression, most likely via the actions of glucocorticoids on the organization of aggression in this species (although a potential role for the adrenal androgen DHEA has not been examined to date). Syrian hamsters initiate aggression on approximately postnatal Day 20, engaging in considerable flank marking (a stereotyped form of communicative behavior employing odors) and play fighting (Goldman and Swanson, 1975; Ferris et al., 1996). Play fighting occurs as soon as coordinated movement is possible, and juvenile hamsters engage in more attacks during agonistic behavior than adult hamsters (Pellis and Pellis, 1998; Wommack et al., 2003). The number of attacks increases over development, reaching peak levels at approximately postnatal Day 35, and subsequently decreases until stable levels are reached at ∼45 days postnatal (reviewed in Delville, 2003).

It is important to note that the development of aggression in Syrian hamsters occurs when juvenile hamsters are attempting to establish independence from other territorial males and thus, are potentially exposed to social stressors from their dominant conspecifics (Delville, 2003). Recent research has examined the role of the stress response, and particularly the activation of the HPA axis and subsequent release of glucocorticoids, in the development of agonistic behavior in juvenile Syrian hamsters (Delville et al., 1998; Wommack et al., 2003). Specifically, juvenile hamsters were placed daily in the home cages of an aggressive adult hamster for short periods from weaning to midpuberty, whereas control animals were moved to an empty cage. Initially, both groups of animals displayed significantly increased levels of circulating cortisol. By two weeks, however, only hamsters exposed to males maintained elevated cortisol levels. Unlike previous studies in rats (Blanchard et al., 1995), chronic social stress did not result in altered body mass or lower circulating T concentrations. Social stress did not affect the frequency of attacks against a smaller intruder during early puberty (Wommack et al., 2003); however, at midpuberty, socially stressed hamsters displayed significantly more attacks than control animals (Wommack et al., 2003). Socially subjugated animals also performed adult-like attacks at an earlier developmental age, while decreasing the amount of play-fighting (Wommack et al., 2003). More recent data provide support for the idea that social stress during development may enhance offensive aggression in Syrian hamsters. Specifically, hamsters were exposed to social subjugation or isolation (control) from postnatal day 28 until midpuberty (Wommack et al., 2004). Hamsters were then assessed under basal conditions or after social defeat during early puberty, midpuberty or early adulthood. Socially stressed animals had lower post-defeat cortisol levels than control males during midpuberty, as well as complete inhibition of olfactory investigation of aggressive adults (Wommack et al., 2004). Collectively, these results suggest an important role for social stress, and subsequent HPA activation, in the development of age-appropriate levels of aggression.

2.5 Neurosteroids and Aggression

The concept of "neurosteroids" was first introduced by Baulieu to describe the high levels of the androgen DHEA, and its sulfated form DHEA-S, seen in rat brain even after castration and adrenalectomy (Baulieu, 1981; Corpechot et al., 1981). It is now established that DHEA, among other steroid hormones [e.g., allopregnanolone (ALLO)], can be synthesized *de novo* within the central nervous system and can act locally on specific neural substrates to regulate behavior (Simon, 2002). For example, a recent study has demonstrated that intermale aggression is associated with changes in brain neurosteroid synthesis (Pinna et al., 2005). Specifically, administration of testosterone propionate (TP) to male mice decreased brain ALLO content by ∼40% and was correlated with increased aggression. Increasing brain ALLO levels pharmacologically attenuated aggressive behavior in these mice. It remains unclear, however, how neurosteroids might be synthesized in adult rodent brain, given that some of the relevant synthetic enzymes (e.g., P450c17) are apparently nondetectable in the brains of many rodents (Mellon and Vaudry, 2001).

DHEA has also been implicated in the regulation of aggression in rodents. For example, DHEA has been shown to be a potent inhibitor of female-typical aggression (Bayart et al., 1986; Haug et al., 1989, 1992; Young et al., 1996; Perché et al., 2000). Attack behavior in ovariectomized females, intact females, or castrated male mice toward lactating females can be significantly reduced following treatment with exogenous DHEA (Bayart et al., 1989; Brain and Haug, 1992). Because this effect requires prolonged (~15 days) DHEA treatment, it has been suggested that the results are consistent with a genomic mechanism of action (Lu et al., 2003). More recently, it has been shown that prenatal T treatment enhances DHEA-induced inhibition of aggression in female offspring (Perché et al., 2001). It is important to note, however, that this testing paradigm (i.e., aggression toward a lactating intruder) is less commonly employed than more traditional models of aggression (e.g., intermale aggression); thus, the role of DHEA in mediating other forms of aggression has received very little experimental attention. One recent finding, however, suggests that increases in aggressive behavior can be induced by a single injection of DHEA-S immediately prior to behavioral testing in mice tested in a neutral arena (Nicolas et al., 2001). Furthermore, treatment with COUMATE, a drug that inhibits the steroid sulfatase enzyme involved in converting DHEA-S to DHEA, also increased aggression in male mice (Nicolas et al., 2001). Despite these intriguing results, more studies are needed to fully elucidate the role of DHEA and DHEA-S in intermale aggression.

The effects of DHEA on aggression have led to an attempt at determining the physiological mechanisms underlying this behavioral response, with much of the work focused on the interaction of this steroid with gamma aminobuteric acid (GABA) neurotransmission (reviewed in Simon, 2002). It has been demonstrated that DHEA alters brain levels of pregnenolone sulfate (PREG-S), a neurosteroid that inhibits GABAergic actions via the $GABA_A$ receptor. Specifically, DHEA-induced changes in GABA activity appear responsible for the effects of DHEA on aggression. These results are consistent with previous findings demonstrating an inhibitory effect of GABA on aggression (Miczek et al., 1994, 1997). Although the precise mechanisms of action are still unknown, DHEA can modulate the actions of both GABA and glutamatergic NMDA receptors (Labrie, 1998; Mellon and Vaudry, 2001). Alternatively, DHEA may act by further conversion in the brain to T and E_2 via 3β-HSD and the aromatase enzyme (Soma et al., 2004).

More recent findings suggest that an additional mechanism for the effects of DHEA on aggression may exist (Simon, 2002). Although is has long been assumed that DHEA is incapable of directly interacting with androgen receptors (AR), recent data suggest that DHEA upregulates AR in mouse brains (Lu et al., 2003). For example, because intact and gonadectomized, T-treated males do not display aggression toward females and prolonged treatment with DHEA is necessary to elicit antiaggressive effects, it has been speculated that androgens such as DHEA play an inhibitory role, presumably through a genomic action by binding directly on AR and altering AR transcription and translation (Simon, 2002). For example, it has recently been demonstrated that DHEA can compete for recombinant AR binding, up-regulate neural AR protein levels in mouse brains and immortalized GT1-7 hypothalamic cells, and induce transcription through AR in CV-1 cells, suggesting direct actions of DHEA on AR (Simon, 2002). Although this idea is intriguing, the affinity of DHEA for AR is low relative to T, so high local levels of DHEA would be necessary for this mechanism to be plausible.

2.6 Arginine Vasopressin and Dominant/Subordinate Relationships

Arginine vasopressin (AVP) when released as a neurohormone from the posterior pituitary is involved in the control of several important homeostatic functions, including the regulation of cardiovascular responses and fluid balance. However, there are also neural circuits in the brain that contain AVP and that have important effects on social behavior, including aggression (Ferris and Delville, 1994; Albers and Bamshad, 1998; Albers et al., 2002). One of the first demonstrations of a role for AVP in agonistic behavior occurred accidentally when it was found that injections of AVP into the anterior hypothalamus (AH) elicited flank marking in Syrian hamsters (Ferris et al., 1984). Since this initial discovery, a number of studies have implicated AVP in the regulation of aggressive behavior and dominance status (Ferris and Delville, 1994; Albers and Bamshad, 1998, Albers et al., 2002). For example, AVP released within the medial preoptic area (MPOA)-AH appears to be critical for the communication of dominance status by flank

marking in Syrian hamsters (Ferris et al., 1986). AVP antagonists injected into dominant hamsters inhibit the high levels of flank marking observed when these hamsters are placed with their subordinate partners. In response to this inhibition of flank marking in the dominant hamsters, subordinate hamsters significantly increase their levels of flank marking. Injections of AVP into the MPOA-AH of subordinate hamsters can also increase flank marking. Although injections of AVP and AVP antagonists can significantly modulate flank marking in both dominant and subordinate hamsters, these injections do not appear to modify the basic dominant/subordinate relationship (Ferris et al., 1986).

Studies on both Syrian hamsters and laboratory rats also suggest a role for AVP in the regulation of offensive aggression, most typically studied using the resident–intruder model. For example, injections of an AVP receptor antagonist into the AH trigger a dose-dependent decrease in offensive aggression of a resident male hamster toward an intruder (Potegal and Ferris, 1990). Similar results were found when animals were tested using a neutral arena. Injection of AVP into the AH increases aggression; however, the concentration of AVP administered must be below the levels that induce flank marking (Ferris et al., 1997; Caldwell and Albers, 2004). In addition, it appears that AVP enhances aggression only in hamsters that have been exposed to social conditions that increase their potential for aggression (see Caldwell and Albers, 2004). There is also evidence that AVP injected into the ventrolateral hypothalamus can facilitate offensive aggression (Ferris and Delville, 1994).

Aggressive behavior plays a critical role in the establishment of dominant/subordinate relationships in rodents. For example, when two male rodents that have not experienced one another previously are placed in a neutral arena, an initial period of high aggression is followed by a decrease in aggression once one male has established dominance. Injection of a specific AVP V_{1a} receptor antagonist into hamsters that have not previously interacted prevents the formation of a dominant/subordinate relationship (reviewed in Albers and Bamshad, 1998), suggesting that the period of aggression or flank marking is necessary for the establishment of social hierarchies. In contrast, injections of either AVP or AVP antagonists in hamsters with established hierarchies can affect the amount of flank marking displayed by both dominant and submissive hamsters. For example, injection of AVP into the MPOA-AH of subordinate hamsters increases flank marking in the presence of the dominant animal. Conversely, injections of an AVP antagonist into a dominant hamster reduces flank marking, despite the presence of the subordinate hamster (reviewed in Albers and Bamshad, 1998). The development of stable dominant/subordinate relationships appears to result in marked changes in the AVP system within the CNS. For example, subordinate hamsters in stable dominance relationships have reduced levels of AVP-ir and fewer AVP-ir fibers in the AH, but not in other brain regions, relative to dominant animals (reviewed in Albers and Bamshad, 1998). Lastly, knock-out mice that lack the V_{1b} AVP receptor display a marked reduction in aggression (Wersinger et al., 2002).

In addition to mediating aggression in adult animals, AVP has been implicated in the *development* of aggression in several rodent species. For example, adult sexually naïve male prairie voles (*Microtus ochrogaster*) are typically not aggressive; however, significant aggression is seen toward strange males within 24 h of mating. Furthermore, icv injections of AVP can induce intermale aggression in sexually naïve prairie voles, mimicking the effects of social experience (Stribley and Carter, 1999). Recently, it has been shown that developmental exposure of sexually naïve male prairie voles to AVP can elicit long-lasting changes in social behavior, with sexual naïve males exhibiting levels of aggression comparable to that displayed by male postmating, suggesting an important role for early AVP in the development of aggression in this species (Stribley and Carter, 1999). The monogamous California mouse (*Peromyscus californicus*) is typically more aggressive than its polygynous relative, the white-footed mouse (*Peromyscus leucopus*). Cross-fostering pups from these two species can reverse the species-specific bias toward aggression, with California mouse pups becoming less aggressive in a resident–intruder test and white-footed mouse pups becoming more aggressive in a neutral arena test (Bester-Meredith and Marler, 2001). Interestingly, the decrease in aggression seen in California mice was associated with decreases in AVP-immunoreactive in the bed nucleus of the stria teminalis (BnST), supraoptic nucleus, and medial amygdala (Bester-Meredith and Marler, 2001). In Syrian hamsters, repeated androgenic steroid treatment during adolescence increases hypothalamic V_{1a} AVP receptor staining and facilitates offensive aggression (DeLeon et al., 2002). Collectively, these studies suggest that in addition to regulating adult aggression, AVP plays an important role in the development of agonistic behavior in a range of rodent species.

3 Neuroendocrine Regulation of Aggression in Birds

Studies in birds have contributed greatly to our current understanding of the neuroendocrine regulation of aggression (Harding and Follett, 1979; Wingfield et al., 1987; Konishi et al., 1989; Schlinger and Callard, 1990; Goodson et al., 2005). Indeed, the first study of hormones and aggressive behavior was conducted in birds, as described below. More recently, field studies of wild songbirds, such as sparrows, have been invaluable for revealing hormone–behavior relationships across species, seasons, and habitats, as well as the molecular mechanisms underlying territorial aggression. Free-living animals are often more aggressive and have higher circulating T levels than captive animals (Wingfield, 1984a, b; Schwabl and Kriner, 1991; Smulders et al., 2000). In addition, testing conditions in the laboratory can have large, unexpected effects on behavior, particularly when animals are forced to interact in small spaces without the opportunity to flee. For these reasons, field studies can be an important complement to laboratory experiments. Such approaches in birds have produced novel insights into the social regulation of T levels, the expression of aggression during the nonbreeding season, the "costs" of elevated T levels, and species-specific effects of hormones (Goodson et al., 2005).

3.1 Aggressive Behavior

Our knowledge of the natural history, ethology, social systems, and seasonal reproductive patterns of birds is by far greater than for other classes of vertebrates. There are approximately 9,000 species of birds, living in habitats as diverse as deserts, arctic tundra, and tropical rainforest (DeVoogd et al., 1993; Brenowitz, 1997). A total of 5,300 species belong to the order Passeriformes ("perching birds"), which is composed of the oscine suborder ("true songbirds," 4,000 species) and the suboscine suborder (1,300 species; e.g., antbirds, flycatchers). There is a rich diversity of social systems from colonial to strictly territorial species, permitting comparative studies of aggression and the underlying physiological mechanisms (Soma and Wingfield, 1999; Goodson et al., 2005). In addition, many birds are terrestrial and diurnal, making their natural behavior relatively easy to observe in the field. Moreover, some species are highly territorial and show little spatial movement, facilitating long-term behavioral studies of individual animals.

For example, the ethologist Nice (1943) carried out groundbreaking studies of song sparrows (*Melospiza melodia*), a common North American songbird, by marking animals with unique combinations of color bands and conducting detailed behavioral observations, sometimes following individuals for several years. Nice (1943) provides descriptions of territorial aggression, including postures and vocalizations. For example, song sparrows use songs, specific threat postures, feather "puffing," wing "waving," and actual physical contact during territorial conflicts. Such encounters can go on for hours or days. Nice (1943) also described seasonal patterns of territorial behavior and the development of such behaviors in juveniles. Moreover, she was able to incorporate these behavioral observations into the larger context, including weather and population ecology. This foundation provides significant advantages when studying the hormonal and neural mechanisms underlying aggressive behavior in song sparrows (see later). Similar data are available for many other species, including European robins (*Erithacus rubecula*), European starlings (*Sturnus vulgaris*), and red-winged blackbirds (*Agealius phoenecius*), in which the neuroendocrinology of aggression has been investigated (Beletsky et al., 1990; Kriner and Schwabl, 1991; Schwabl and Kriner, 1991; Pinxten et al., 2002).

3.2 The Role of Testicular Hormones: Berthold's Capons

The study of hormones and aggression, and of hormones in general, can be traced back to the work of Berthold (1849). Berthold removed the testes of immature male chickens (the castrated animals are called capons) and found a decrease in some secondary sex characteristics and male-typical behaviors. Specifically, capons did not crow, did not try to mate with females, and did not fight aggressively with other males. Importantly, transplantation of testes into castrated animals restored male sexual and aggressive behavior. When a single autotransplanted testis was replaced after all original vascular and neural connections to the

testis were cut, these animals showed normal male aggressive behavior. Further, when a single heterotransplanted testis was replaced, these animals also showed typical male fighting. Additionally, when the testis from one heterotransplanted bird was removed, the animal behaved as a capon. Upon dissection, Berthold found that the transplanted testes in the abdomen had established new vascular connections. From these results, Berthold concluded that the testes release a substance into the blood that affects behavior and the body in general. Thus, from its beginning, the study of hormones and aggression was focused on gonadal secretions, primarily T. This conceptual framework has greatly influenced subsequent studies up to the present day. It is clear that T secreted by the testes is important for the expression of aggressive behavior. However, an *exclusive* focus on the testes and circulating levels of T is too simplistic and can often be misleading.

In seasonally breeding avian species, the gonads grow (recrudesce) markedly prior to the breeding season and regress following the termination of breeding. In parallel, circulating sex steroids fluctuate, generally with high levels during the breeding season and basal levels during other times of the annual cycle (e.g., molt, nonbreeding season) (Farner and Wingfield, 1980; Wingfield and Farner, 1993). In many species, territorial behavior shows a similar seasonal pattern: animals are territorial in spring, but gregarious and form flocks in autumn and winter (Wingfield et al., 1997, 2001). Note, however, that there are exceptions to this general pattern, and such exceptions can yield novel insights into the neuroendocrinology of aggression (see Section 3.6 and also Section 2.2).

3.3 Field Endocrinology and the Challenge Hypothesis

Ethologists generally focus on the behavior of free-living birds, whereas early behavioral endocrinologists focused on domesticated species, such as chickens. These two fields were combined in a series of elegant studies of wild songbirds by Wingfield and colleagues (Wingfield et al., 1987, 1990). Previous laboratory studies had demonstrated that long day lengths stimulate testis growth and increase plasma T levels in birds, but field studies were important for identifying the role of social interactions. In field studies, the temporal pattern of T titers differed from lab studies, and the absolute values of T concentrations were higher in wild-caught animals (Wingfield, 1984a, b). These two discrepancies suggested that environmental information in addition to day length was critical for T secretion.

As mentioned previously, song sparrows are common North American songbirds. In a migratory population, plasma T levels are very high in free-living males when they first arrive in early spring and are aggressively establishing breeding territories (Wingfield, 1984a, b). After pairing with females, plasma T levels in males decline, even though day lengths are increasing at this time. These data raised the hypothesis that aggressive male–male interactions stimulate T secretion, which may be important during periods of intense fighting or challenge. This hypothesis was tested experimentally in both the field and laboratory. In the field, wild territorial males were given either long-term subcutaneous T implants or empty implants in spring (Wingfield, 1984a, b). T-treated males were more aggressive, as measured by simulated territorial intrusions (STI) using taped song playback. Hormone levels were measured in the untreated *neighbors* of implanted animals. The immediate neighbors of T-treated males had higher circulating T levels than the immediate neighbors of control subjects. The hormone levels of distant neighbors were not affected. These results suggest that cues from aggressive males can stimulate T secretion (Wingfield, 1984a, b). In a separate field experiment, free-living males were exposed to a simulated territorial intrusion (STI) using both song playback and a live caged decoy for up to 120 min (Wingfield, 1985). The combined visual and auditory cues provoked a robust aggressive response. In addition, plasma T levels were significantly higher in males exposed to STIs than in controls. Further field experiments demonstrated that rapid changes in plasma T can occur within only 10 min of STI exposure (Wingfield and Wada, 1989).

In one laboratory study, the timecourse and specificity of STI response were investigated (Wingfield and Wada, 1989). Breeding male song sparrows were captured in the field and brought into captivity. After interaction with a novel male conspecific, plasma T titers were elevated. Interaction with a control stimulus (i.e., heterospecific male) had no effect. Lab experiments also assessed the relative importance of auditory

and visual cues using taped song playback only or a devocalized male only. Exposure to the devocalized male significantly increased plasma T, whereas there was only a trend for the song playback to do so (Wingfield and Wada, 1989).

These results and studies in many other bird species led to the formulation of the "challenge hypothesis" (Wingfield et al., 1990). This hypothesis states that plasma T levels and aggression are positively correlated during periods of social instability, or challenge, such as establishment of territorial boundaries or attempts at territory takeover. During such periods, aggressive interactions are more frequent, leading to high T concentrations. In contrast, during times of social stability, status or boundaries are maintained by social inertia, and plasma T levels are lower. Under such stable conditions, aggression and plasma T may not be correlated. This idea has been extremely influential and has been examined in a variety of birds, fish, lizards, rodents, and primates.

It may be advantageous to limit high circulating T levels to periods of instability during the breeding season because high T levels incur "costs" (Ketterson et al., 1992; Ketterson and Nolan, 1999). For example, systemic T treatment can increase the metabolic rate (Wikelski et al., 1999) and decrease body mass and fat stores in birds (Wingfield, 1984a, b; Ketterson et al., 1991). In addition, T treatment suppresses the immune function in some, but not all, species of birds (Casto et al., 2001; Owen-Ashley et al., 2004). These costs of high circulating T are likely to be most evident in the field, where food is not typically available ab libitum and parasites are often present. Moreover, several field studies have shown that T treatment can reduce male parental care (Hegner and Wingfield, 1987; Ketterson et al., 1992). In many bird species, biparental care is important, or even obligatory, for offspring survival. In such species, there should be strong evolutionary selection for low T levels during the parental phase of the breeding season (Van Roo, 2004). The relative importance of the costs of T will likely change across different stages of the life history cycle (e.g., breeding versus nonbreeding season) and across different habitats (e.g., temperate zone versus tropics) (Levin and Wingfield, 1992; Goymann et al., 2004).

3.4 Possible Neural Sites of Action of Testosterone

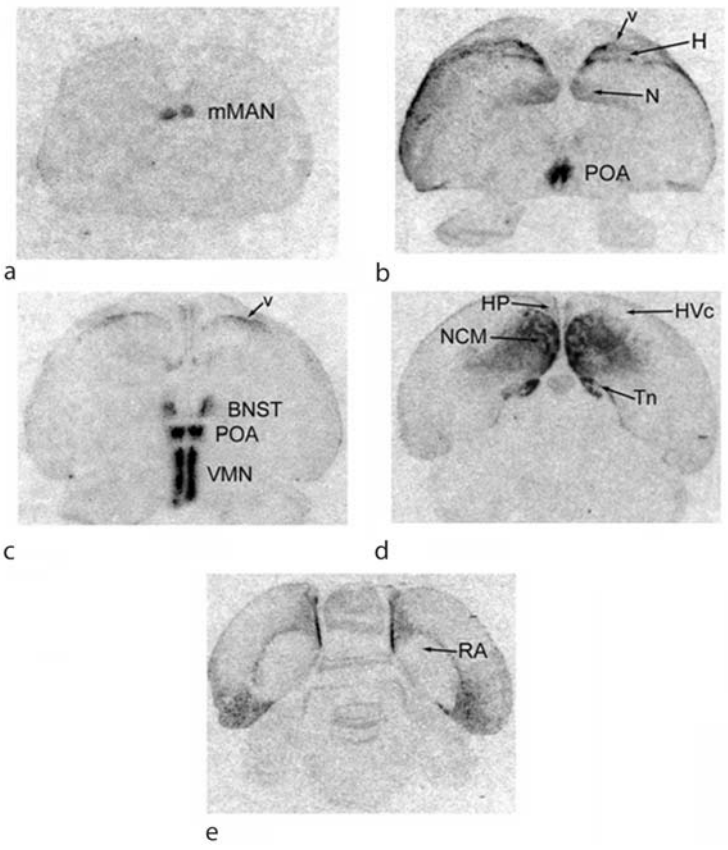
Where does T act in the avian brain to modulate aggression? In an important early study, Barfield et al. (1978) used steroid autoradiography and injected radiolabeled T into adult (3–4 months old) male chickens. The subjects were castrated at 5 weeks of age to remove gonadal T that would compete with radiolabeled T for receptors (Barfield et al., 1978). This method does not distinguish between direct uptake of T and uptake after local metabolism of T to 5α-DHT, E_2 or other steroids. Other caveats are that castration can affect the expression of hormone receptors, and castration will not reduce the competition of possible nongonadal androgens with radiolabeled T for receptors. Nevertheless, the results demonstrated that radioactivity accumulated in the POA (*Figure 8-3*), several regions of the hypothalamus, nucleus taeniae (Tn, homologous to the mammalian medial amygdala), lateral septum, and midbrain (including the central gray) (Barfield et al., 1978). This pattern was similar to that of other vertebrates and coincided with regions known to be important for reproduction. Several of these interconnected regions are considered part of a general "social behavior network" in vertebrates (Wood, 1997; Goodson et al., 2005).

More recent studies have examined the localization of androgen (AR) and ER in the avian brain using immunocytochemistry and in situ hybridization (Gahr et al., 1993; Soma et al., 1999; Fusani et al., 2000; Kim et al., 2004). In addition to the steroid autoradiography results, recent studies demonstrate that there is robust expression of AR and ERα in the BnST, a region involved in regulating aggression (Saldanha and Coomaralingam, 2005). There is less information regarding the expression of ERβ in the avian brain, but ERβ mRNA is present in the avian amygdala and POA (Bernard et al., 1999). Currently, there is no clear evidence for membrane AR or ER in avian brain, and this is a key topic for future research.

It is important to note that songbirds, unlike nonsongbird species such as chickens, have additional sites of abundant AR and ER expression in the song control system, which controls the production of birdsong in aggressive and sexual contexts (Arnold et al., 1976; Brenowitz et al., 1997; Schlinger, 1997; Tramontin and Brenowitz, 2000). The song control system is a circuit comprising interconnected, discrete regions that

Figure 8-3

Rostral (a) to caudal (e) distribution of brain aromatase mRNA from autoradiographic film from a wild male song sparrow in spring. (a) Expression in the medial magnocellular nucleus of the anterior nidopallium (mMAN). (b) Expression in the hyperpallium (H) near the ventricles (v), the nidopallium (N), and preoptic area (POA). (c) Expression in the bed nucleus of the stria terminalis (BNST), POA, and ventromedial nulceus (VMN). (d) Expression in caudomedial nidopallium (NCM), nucleus taeniae of the amygdala (Tn), and hippocampus (HP). A few cells in caudomedial HVC expressed aromatase. (e) Expression in the caudal nidopallium, but not in the robust nucleus of the arcopallium (RA)



controls the muscles of the syrinx (the vocal production organ in birds) (Nottebohm et al., 1976; Brenowitz et al., 1997). Several telencephalic song nuclei, such as HVC, robust nucleus of the arcopallium (RA), and Area X of the striatum, contain AR (Arnold et al., 1976; Harding et al., 1984; Soma et al., 1999; Kim et al., 2004) (*Figure 8-3*). HVC also contains ERα (Walters and Harding, 1988; Gahr et al., 1993; Perlman and Arnold, 2003; Saldanha and Coomaralingam, 2005). No song nuclei are known to express ERβ (Bernard et al., 1999).

In addition to localizing hormone receptors, a complementary approach is to assess the effects of local, intracerebral hormone implants in castrated animals. Small TP implants into the POA-AH do not activate aggression in chickens, but are effective in ring doves (Barfield, 1971; Phillips and Barfield, 1977). TP implants near Tn or the lateral septum do not increase agonistic behavior in chickens or ring doves (Barfield, 1971). It remains possible, however, that *multiple* sites must be activated by T to elicit aggression. Administration of hormone receptor antagonists locally in intact animals can be used to test this idea.

3.5 Brain Aromatase and Aggression

In many cases, the effects of plasma T are mediated by local metabolism within the brain. For example, T can be metabolized to 17β−E_2 by the enzyme P450 aromatase or to 5α-DHT by 5α-reductase (Schlinger and Callard, 1987; Fusani et al., 2000; Balthazart et al., 2003). Importantly, aromatase is expressed at high levels in the avian brain, facilitating its measurement in birds relative to mammals. 5β-reductase metabolizes T to 5β-DHT, an apparently inactive steroid, and brain 5β-reductase is present at very high levels in birds, but not rats and mice (Schlinger and Callard, 1987).

A series of laboratory studies with breeding Japanese quail (*Coturnix coturnix japponica*) illustrates the key role of brain aromatase in the expression of male aggressive behavior (Schlinger and Callard, 1990). To assess aggressive behavior, males were allowed to view a stimulus bird (i.e., nonaggressive female) through a glass partition (Schlinger and Callard, 1989). The number of pecks directed toward the glass partition and locomotor activity were recorded for 2 min. Males were killed either 90 s or 24 h after behavioral tests to measure aromatase, 5α-reductase, and 5β-reductase activities in several brain regions. Enzyme activities were assessed by measuring the metabolism of radiolabeled androgen (androstenedione, AE) by tissue homogenates in vitro. Aggressive behavior was positively correlated with aromatase activity in the hypothalamus but not in other brain regions (Schlinger and Callard, 1989; Schlinger and Arnold, 1992). Aggressive behavior was not correlated with 5α- or 5β-reductase or circulating T or estrogen levels. In a separate study, aggression was correlated with nuclear ("occupied") ER in the hypothalamic-POA (Schlinger and Callard, 1989). Moreover, in breeding males, experimental treatment with an aromatase inhibitor or ER antagonist decreased aggression, but a 5α-reductase inhibitor and AR antagonist had no effect (Schlinger and Callard, 1990). Taken together, these data argue that T affects aggression in quail via the aromatase pathway. In addition, there is evidence for a negative correlation between aggression and 5β-reductase activity in quail (Delville et al., 1984).

Generally similar results were obtained in studies of zebra finches (*Taeniopygia guttata*) and red-winged blackbirds. Captive reproductively active male zebra finches were castrated and treated with AE (androstenedione; an aromatizable androgen), AE + an aromatase inhibitor (ATD), or AE + ATD + E_2 (Walters and Harding, 1988). Although levels of aggression in this social species were generally low, only the AE and AE + ATD + E_2 groups displayed aggression, suggesting that the aromatase inhibitor reduced aggression (Walters and Harding, 1988). In captive breeding male red-winged blackbirds, castration greatly reduced aggression. Treatment of castrated animals with aromatizable androgens (T or AE) or E_2 + 5α-DHT in combination restored aggression (Harding et al., 1988). Treatments with E_2 alone or 5α-DHT alone were not effective, suggesting that the presence of both metabolites was necessary for the full expression of aggression (Harding et al., 1988). In a separate study of captive red-winged blackbirds, treatment with an AR antagonist (flutamide) decreased dominance and aggression, but not singing (Searcy and Wingfield, 1980). In a field experiment, blackbirds treated with flutamide and an aromatase inhibitor in combination lost parts of their territories (Beletsky et al., 1990). These data suggest that both estrogenic and androgenic metabolites of T are important for aggression in some avian species.

Field studies have examined seasonal changes in brain aromatase and/or 5α- and 5β-reductase in wild birds. In pied flycatchers (*Ficedula hypoleuca*) in Sweden, during early summer, males switch from defending a territory to helping with parental care. At the same time, plasma T levels decrease, and aromatase activity and immunoreactive cells in the diencephalon decline (Foidart et al., 1998). In another study of this species, aggression was measured using a simulated territorial intrusion, or STI; males were captured immediately afterwards; and brain tissue was collected (Silverin et al., 2004). Aromatase activity was measured by the release of tritiated water during aromatization of [1β-^{3}H]-AE, which is a sensitive technique but one that does not assess 5α- or 5β-reductase. Aggression was not correlated with plasma T or 5α-DHT. Aggression was, however, positively correlated with aromatase activity in the POA-anterior diencephalon, but not in the posterior diencephalon or telencephalon (Silverin et al., 2004). Specific telencephalic regions were not examined (e.g., Tn, BnST, lateral septum). It is possible that the STI rapidly upregulates aromatase activity, and this is a topic for future studies.

In Lapland longspurs (*Calcarius lapponicus*) in arctic Alaska, during the brief breeding season, males rapidly court females, then aggressively guard mates, and finally switch to parental care. Aggression is only

robustly expressed during the mate-guarding phase. In this species, plasma T is correlated with singing behavior but not territorial aggression, and T treatment increases song but not aggression (Hunt et al., 1995, 1997). Aromatase, 5α-, and 5β-reductase activities were measured in seven brain regions during all three phases of the breeding season (Soma et al., 1999). Aromatase activity in the anterior diencephalon generally matched temporal changes in aggressive behavior. Changes in 5β-reductase did not explain the pattern of aggressive behavior (Soma et al., 1999). Taken together, these data suggest a significant role of aromatase in the anterior diencephalon/POA in the control of aggressive behavior. In addition, studies in song sparrows suggest the presence of aromatase in Tn and perhaps BnST is involved in nonbreeding territoriality (see Section 3.6).

3.6 Territorial Aggression During the Nonbreeding Season: Beyond Berthold

During the autumn and winter, many birds are in nonbreeding condition, which is generally characterized by regressed gonads and secondary sex characteristics (e.g., cloacal protuberance, wattles) and basal plasma sex steroid levels. Typically, nonbreeding birds abandon exclusive territories in favor of flocks. Some species, however, aggressively defend territories during the nonbreeding season, even if plasma T is basal at this time (Wingfield et al., 1997; Soma and Wingfield, 1999). The physiological regulation of breeding territoriality has been the focus of numerous studies (Wingfield et al., 1987), but the proximate mechanisms underlying nonbreeding territoriality have been enigmatic.

Winter territoriality may be dissociated from plasma T because of the costs of circulating T. In seasonally breeding birds, sex steroids in the general circulation may have particularly high costs during the nonbreeding season. The nonbreeding season may last the majority of the year and can be a difficult time because of low ambient temperatures and reduced food supply (Soma and Wingfield, 1999). Moreover, diurnal birds do not forage during the long nights of winter. Many costs of T during the nonbreeding season may be energetic in nature. For example, T treatment can increase the basal metabolic rate (Wikelski et al., 1999). T treatment also decreases fat stores (Owen-Ashley et al., 2004), which are important for surviving snow and ice storms. T treatment stimulates secondary sex characteristics (Soma et al., 2002), which require energy to grow and maintain. Other costs of T are not directly related to energetic constraints. Importantly, T can suppress the immune system of birds (Casto et al., 2001); T also inhibits molt (Schleussner and Gwinner, 1989). Finally, T treatment may stimulate reproductive behavior, which is inappropriate during the winter (Logan and Carlin, 1991). These various effects may explain why T treatment of wild birds reduces overwinter survival in some species (Dufty, 1989; Moss et al., 1994; Ketterson et al., 1996).

In Washington State, song sparrows (*Melospiza melodia morphna*) are sedentary. Males are territorial during the spring (breeding season), when plasma T is high (Wingfield and Hahn, 1994; Wingfield and Soma, 2002). After breeding, the animals molt their feathers (August–September), and during the molt, plasma T levels are basal, gonads are regressed, and aggression is greatly reduced. Following completion of the molt, there is a resurgence of territorial aggression in the autumn (nonbreeding season). Males in autumn and spring respond similarly during the STI, but autumn birds are less "persistent" after the intrusion has ended. After the decoy is removed, territorial behavior is extinguished more rapidly in autumn (Wingfield, 1994). The social context of territoriality also changes seasonally. Spring territories are defended by a breeding pair, but autumn territories can be defended by individuals, pairs, or larger groups (Wingfield and Monk, 1992; Wingfield, 1994). Plasma T is nondetectable during the autumn, and the testes and cloacal protuberance are fully regressed (Wingfield and Hahn, 1994; Soma and Wingfield, 1999). While agonistic interactions between males increase plasma T in spring (Section 3.3; Wingfield and Hahn, 1994), they do not affect circulating T in autumn (Wingfield and Hahn, 1994; Soma and Wingfield, 2001). Plasma 17β-E_2, 5α-DHT, AE, and estrone concentrations are also basal in males during the nonbreeding season (Soma and Wingfield, 1999). Moreover, castration does not decrease aggressive behavior in autumn (Wingfield, 1994). Thus, aggression may be independent of T in autumn.

The hypothesis that nonbreeding season aggression is independent of T was tested in three field experiments by treating wild song sparrows with pharmacological inhibitors of aromatase, with or without

an AR antagonist. A combined treatment of an aromatase inhibitor (ATD) and AR antagonist (flutamide) decreases nonbreeding aggression in free-living males after 30 days, but not after just 7 days (Soma et al., 1999). Fadrozole, a more potent and specific aromatase inhibitor than ATD, strongly decreases nonbreeding aggression within 10 days. The effects of fadrozole are rescued by E_2 replacement (Soma et al., 2000). Moreover, fadrozole did not affect body condition or plasma CORT levels, indicating that the animals were not affected in a nonspecific manner (Soma et al., 2000). Additional studies suggest that fadrozole can reduce some aspects of autumnal aggression within only 24 h (Soma et al., 2000). These data indicate that sex steroids, particularly estrogens, are important for the expression of aggressive behavior in the nonbreeding season, even though plasma sex steroids are nondetectable and castration has no effect.

In addition, studies have also examined regional and seasonal differences in song sparrow brain aromatase using biochemical and molecular techniques. In situ hybridization reveals that aromatase mRNA is highly expressed in the POA, ventromedial nucleus of the hypothalamus, Tn (avian medial amygdala), BnST, caudomedial nidopallium (NCM, implicated in song perception), and medial magnocellular nucleus of the anterior nidopallium (MMAN, a song nucleus) (Soma et al., 2003). In addition, brain regions were dissected and androgen-metabolizing enzyme activities were measured in several brain regions during spring, molt, and autumn. Aromatase activity in the ventromedial telencephalon (includes Tn) is specifically reduced during molt, matching seasonal changes in aggression (Soma et al., 2003). Aromatase activity in the diencephalon, however, is high only during spring. 5β-reductase is not elevated during molt and thus cannot explain the low aggression during molt. These results suggest that changes in aromatase activity may regulate seasonal changes in aggression in birds.

Despite the evidence implicating aromatization of androgens, the precise source of androgen substrate for brain aromatase in the nonbreeding season remains unknown, because plasma levels of aromatizable androgens (e.g., T and AE) are basal at this time. One endocrine candidate, DHEA, is considered an "inert" androgen precursor and does not bind to AR or ER with high affinity (Kroboth et al., 1999). DHEA, however, can be converted into active sex steroids within tissues that express the appropriate enzymes (Labrie et al., 2001). Interestingly, plasma levels of DHEA are detectable and elevated (several-fold higher than plasma T) in nonbreeding song sparrows (Soma and Wingfield, 2001). Circulating DHEA may originate from the adrenals or regressed testes in autumn (Soma and Wingfield, 2001). As described for rodents, another, as yet untested, possibility is that the brain synthesizes DHEA de novo from cholesterol in the autumn. Across the annual cycle, circulating DHEA levels are specifically reduced during the molt, the one life-history stage when song sparrows show reduced aggressiveness (Soma and Wingfield, 2001). In a separate experiment, treatment of wild nonbreeding males with high physiological levels of DHEA increased territorial singing behavior (but not other territorial behaviors) and the size of the song-control nucleus HVC (Soma et al., 2002). DHEA treatment did not, however, stimulate the growth of a peripheral secondary sex characteristic (cloacal protuberance). More recent results suggest that DHEA treatment, unlike T, does not inhibit the immune system of nonbreeding song sparrows (Owen-Ashley et al., 2004).

Next, DHEA metabolism was examined in song sparrow and zebra finch brain. The in vitro assay measures the conversion of tritiated DHEA to AE and estrogens by the sequential activities of 3β-HSD and aromatase (Soma et al., 2002; Soma, 2004; Soma et al., 2004). The song sparrow brain can indeed convert DHEA to androgens and estrogens, with the highest levels of 3β-HSD activity in the diencephalon and telencephalon (K. Soma, D. Wacker, J. Wingfield, B. Schlinger, unpublished results). Interestingly, seasonal studies demonstrate that neural DHEA metabolism is highest in the nonbreeding season (unpublished results). These data support the novel hypothesis that in nonbreeding song sparrows, adrenal and/or gonadal DHEA synthesis is coupled with neural DHEA metabolism to supply sex steroids to brain circuits controlling aggression. This mechanism would reduce the exposure of peripheral tissues to T, which is deleterious in winter. Thus, nonbreeding song sparrows may circumvent many costs of circulating T. These studies on song sparrows provide a broad picture of hormone–behavior relationships in this species, integrating field studies of behavior, endocrinology, and ecological constraints with biochemical and molecular mechanisms (Wingfield and Soma, 2002).

Similar results have been obtained in field and laboratory studies of tropical birds. Several avian species that breed in the tropics defend territories year-round and have very low levels of circulating sex steroids throughout the year (Levin and Wingfield, 1992; Goymann et al., 2004). One example is the spotted antbird

(*Hylophylax n. naevioides*) in Panama. In this species, both sexes sing and aggressively defend territories year-round in the rainforest (Wikelski et al., 2000). Wild spotted antbirds have basal plasma T and E_2 levels, even during the breeding season, except for transient increases during territorial encounters (Wikelski et al., 1999). In a laboratory experiment, a combined treatment of aromatase inhibitor and AR antagonist decreased male aggressive vocalizations (songs and "snarls") in the breeding season, even though plasma T was basal (Hau et al., 2000). In the nonbreeding season, both sexes show high levels of aggression, primarily to intruders of the same sex (Hau et al., 2004). Both males and females have regressed gonads and low levels of plasma sex steroids in the nonbreeding season (Hau et al., 2004). However, plasma levels of DHEA are elevated in both sexes during the nonbreeding season, and in males, plasma DHEA levels are positively correlated with aggressive vocalizations and/or the duration of territorial intrusions (Hau et al., 2004). These data suggest that DHEA regulates aggressive behavior, particularly vocal behavior, in both males and females of this species. In tropical species facing a large number of parasites, the immune function may be particularly important (Martin et al., 2004), thus favoring the evolution of endocrine mechanisms which avoid the immunosuppressive effects of circulating T.

Taken together, such studies suggest that nonbreeding aggression is not regulated by gonadal T in some species. Instead, circulating DHEA, from the adrenal glands or regressed gonads, may be converted to active androgens and estrogens locally within the brain. It is also possible that DHEA and/or sex steroids are synthesized de novo from cholesterol by the brain (Corpechot et al., 1981). These alternate mechanisms have important consequences for interpreting data on peripheral levels of T and the effects of castration.

Such mechanisms are likely to be of general importance in birds and other vertebrates. Winter territoriality is not uncommon among birds. For example, excellent field studies have documented nonbreeding territories in mockingbirds (Logan and Wingfield, 1990), willow tits (Silverin et al., 1984), European robins (Kriner and Schwabl, 1991; Schwabl, 1992), and European stonechats (Gwinner et al., 1994), to name just a few cases. In these species, robust territorial aggression is expressed despite basal circulating T levels during the autumn and winter. In European robins, an AR antagonist (flutamide) decreases aggression in spring but not in winter (Schwabl and Kriner, 1991). In European stonechats, a combined treatment of flutamide and an aromatase inhibitor (ATD) for 7 days does not decrease aggression in winter (Canoine and Gwinner, 2002). It would be useful to examine the effects of a more potent aromatase inhibitor, such as fadrozole, in this species. Red grouse in Scotland also defend territories in autumn, but in this species, there is a small peak in plasma T during autumn (Mougeot et al., 2005). Thus, the regulation of territorial behavior outside the breeding season is not identical in all species. In addition, birds that do not defend a winter territory may nonetheless display aggressive behavior within dominance hierarchies in winter flocks, for example, European starlings and dark-eyed juncos (Ketterson, 1979; Pinxten et al., 2000; Pinxten et al., 2002; Pinxten et al., 2003). The endocrine mechanisms regulating dominance in flocks are largely unknown.

3.7 Testosterone and Aggression in Juvenile Birds

Several interesting studies have documented the presence of steroids in egg yolk and consequences for behavioral development. For example, in an important study, it was demonstrated that yolk has significant quantities of AE, 5α-DHT, and T in the eggs of captive canaries (*Serinus canaria*) (Schwabl, 1993). In addition, the concentration of T depended on the order in which the eggs were laid. The T content increased with laying order (i.e., the first egg in a clutch had the lowest T levels). Moreover, the social rank of siblings was correlated with the T content in the yolk. These and other data suggest that steroid exposure in ovo can affect subsequent behavior (Whittingham and Schwabl, 2002). Manipulation of steroids in yolk is easier than manipulations of pregnant mammals, making birds excellent animal models for studying the organizational effects of steroids.

In addition to maternal steroids from yolk, young chicks can synthesize steroids independently. For example, black-headed gull (*Larus ridibundus*) chicks defend territories from conspecifics and transiently increase plasma T levels (Ros et al., 2002). An extreme form of aggression in juvenile birds is siblicide. Lethal attacks against siblings have been reported in several avian species. For example, in a

seabird such as the Nazca booby (*Sula nebouxii*), each clutch has 2 eggs that hatch 4–7 days apart (Ferree et al., 2004). If both eggs hatch, the first chick attacks the second chick and aggressively pushes it out of the nest. The second chick then dies of starvation or predation. A recent study indicates that plasma T levels are generally basal in chicks (~0.04 ng/ml), but transiently elevated during aggressive interactions with siblings (up to 0.15 ng/ml) (Ferree et al., 2004). This was true for both first and second chicks. However, even these peaks of plasma T are far lower than typical levels in many breeding adult birds. Plasma DHEA levels were much higher (0.5–4.0 ng/ml) but did not differ among groups. In general, little is known regarding the control of aggression in developing birds, and this remains an excellent topic for future studies.

3.8 Corticosterone and Aggression

In birds, the predominant circulating glucocorticoid is CORT. In song sparrows, aggressive interactions do not appear to increase plasma CORT levels (Wingfield, 1985). However, treatment of wild males with exogenous CORT during the breeding season affected territorial aggression (Wingfield and Silverin, 1986). After 18–24 h of treatment, the majority of CORT-treated males failed to respond to an STI, and the ones that responded did so weakly (Wingfield and Silverin, 1986). Surprisingly, CORT treatment did not affect luteinizing hormone (LH) levels and only decreased plasma T levels slightly.

A similar study was conducted on tree sparrows (*Spizella arborea*) breeding in arctic Alaska (Astheimer et al., 2000). Interestingly, CORT treatment had no effect on aggressive behavior or reproductive hormones in tree sparrows. It is possible that ecological constraints, such as a short breeding season, reduce the sensitivity of neural circuits to CORT, allowing animals to successfully breed in spite of environmental stressors. This could be achieved, for example, by modulating corticosterone binding globulin (CBG) or glucocorticoid receptor levels (Wingfield and Sapolsky, 2003).

3.9 Arginine Vasotocin and Aggression

Arginine vasotocin (AVT) is a neuropeptide homologous to AVP in mammals. Classically, AVT/AVP neurons in the hypothalamus have been shown to play an important role in water balance. More recently, AVT/AVP neurons in the extended amygdala (medial amygdala and BnST) have been implicated in the regulation of social behavior (Goodson and Bass, 2001). These neurons project to many regions, including the lateral septum, nucleus accumbens, and periaqueductal gray (Goodson and Bass, 2001). In particular, the lateral septum receives AVT input from the BnST and contains high levels of AVT/AVP receptors.

In songbirds, a series of studies by Goodson and colleagues have examined the behavioral effects of septal AVT infusions in different species. Interestingly, intraseptal AVT administration had opposite effects in species with different social systems. In a colonial species, the zebra finch, AVT infusions facilitated aggressive behavior (Goodson and Adkins-Regan, 1999). In contrast, in territorial species, the violet-eared waxbill (*Uraeginthus granatina*) and field sparrow (*Spizella pusilla*), AVT infusions inhibited overt physical aggression (Goodson, 1998a, b). Zebra finches and violet-eared waxbills are both estrildid finches and share critical features of breeding ecology, except for social organization. Thus, this species comparison is less likely to be confounded by phylogeny or other ecological variables.

Song sparrows have also been a useful model system for understanding the role of AVT in territorial aggression. In one experiment, immediate early gene (ZENK) responses were assessed following STI and/or nonsocial stress (restraint) in breeding male song sparrows under semi-natural conditions. Exposure to STI and/or restraint significantly increased ZENK protein in the lateral septum, whereas the medial BnST showed a highly selective response to STI. Interestingly, infusion of an AVT/AVP antagonist (Manning compound, specific to V_1 receptors) into the lateral ventricle abolished these ZENK responses, and influenced ZENK-ir in the medial BnST only after STI, but not after restraint (Goodson and Evans, 2004). These data suggest that AVT acts within the BnST to specifically modulate neural responses to social stimuli. In a second experiment, the role of DHEA in nonbreeding song sparrows was examined. Captive male song sparrows were exposed to an STI and then perfused 90 min later. Plasma DHEA levels were

negatively correlated with immediate early gene expression in medial BnST (J. Goodson, K. Soma, unpublished results). In addition, plasma DHEA levels were *positively* correlated with AVT cell counts in the medial BnST, which may indicate decreased transport and release of AVT (unpublished results). Although speculative, one possible interpretation of these data is that DHEA acts within the medial BnST to decrease the transport and release of AVT in the lateral septum. Because intraseptal AVT inhibits aggressive behavior in territorial species, DHEA would be reducing a "brake" on aggression.

4 Neuroendocrine Regulation of Aggression in Primates

Aggression has been a central topic of primate research for decades. A great deal of research indicates the importance of environmental and social influences on the development and expression of primate aggressive behavior. Dominance is certainly the most well-known social influence on aggression, and dominance hierarchies are considered a major organizing principle in many primate societies. Knowing an animal's dominance rank provides valuable information about that individual's aggressive tendencies. In primates, high-ranking males are more likely to initiate and win aggressive encounters, but they are not necessarily the most aggressive animals in the group (Bernstein et al., 1983). Furthermore, unlike in rodents, in primates individual aggressive power does not necessarily correlate with dominance rank. For instance, the best fighters do not inevitably achieve the highest dominance rank because alliances are critical for acquiring and maintaining high dominance rank (Chapais, 1992). Young primates must learn the proper settings for expressing aggression and the social skills required for recruiting allies. In sum, achieving high dominance rank in primate species probably depends as much on inhibiting aggression as it does on expressing aggression.

Although the study of the hormonal mechanisms of aggression has some experimental attention, much less is known than in the case of rodents. Most recent studies on the neuroendocrine regulation of primate aggression have used fecal or urinary assays to measure androgens. Although the advent of these assays has stimulated new research questions in primate socioendocrinology, their limitations need to be recognized. The different steroids and steroid metabolites present in urine and feces are species-specific, and the levels that are excreted frequently represent a fraction of plasma concentrations. The extended time tag for fecal excretion probably favors the use of fecal samples, and to a lesser extent urinary samples, for assaying hormonal baselines; however, this characteristic makes fecal steroids a less-sensitive measure of hormonal responses to a single behavioral event. Also, most immunoassay kits used to detect steroids were developed for use with human serum or plasma, and may not have been tested for cross-reactivity with metabolites that may be present in urine or fecal samples. High cross-reactivity between excreted metabolites can limit conclusions regarding the origin of a steroid and its mechanism of action. In addition, plasma, urinary, and fecal assays are not recommended for investigating a steroid's mechanism of action due to the importance of local metabolism. Although most primate studies report little cross-reactivity between steroids and related metabolites, some studies have reported considerable cross-reactivity. For instance, 46% cross-reactivity between T and DHT was reported in a study using urine samples in chimpanzees (*Pan troglodytes*) (Muller and Wrangham, 2004), which makes conclusions regarding specific hormone–behavior relationships difficult. Finally, most research on the neuroendocrinology of primate aggression has been conducted with Old World monkeys such as macaques, baboons, and vervets. Apes, prosimians, and New World monkeys share some characteristics with Old World monkeys, but unfortunately much less is known about the neuroendocrine mechanisms regulating aggression in these primates. Thus, our review is biased toward Old World monkeys, although we include examples from other primate taxa, including humans, when possible.

4.1 Androgens and Aggression

In general, high rates of aggression tend to be positively correlated with elevated androgen concentrations in nonhuman primates. This correlation is observed in seasonal changes in androgens and rates of aggression, sex differences in aggression, and increased aggression at puberty. Many primates are seasonal breeders and these species have provided much of the information available on correlations between androgens and

aggression. Seasonality in testicular activity, with circulating T levels peaking during the mating season, has been reported in many primate species, including squirrel monkeys (*Saimiri sciureus*: Wiebe et al., 1988), Hanuman langurs (*Presbytis entellus*: Lohiya et al., 1998), ringtailed lemurs (*Lemur catta*: Cavigelli and Pereira, 2000), mandrills (*Mandrillus sphinx*: Setchell and Dixon, 2001), and several species of macaques (*Macaca mulatta*: Gordon et al., 1976; *M. radiata*: Glick, 1979; *M. fuscata*: Barrett et al., 2002). Both environmental and social cues may elicit the onset of increased T production. Some studies have shown that environmental cues, such as photoperiod, are sufficient to produce seasonal fluctuations in T secretion (Rostal et al., 1986; Perret, 1992; Herndon et al., 1996). In contrast, other studies have found that exposure to females is necessary to maintain a seasonal pattern of T secretion (Gordon et al., 1978; Schiml et al., 1996), and that exposure to sexually active females outside the mating season can elicit increases in T (Ruiz de Elvira et al., 1982). Several studies have shown that rates of aggression in males tend to parallel the rise in T during the mating season (Gordon et al., 1976; Rostal et al., 1986; Cavigelli and Pereira, 2000). In addition, seasonal changes in T have been associated with aggression in females. In ringtailed lemurs, aggressive conflicts between females were found to increase during the mating season and correspond to a seasonal increase in fecal androgen concentrations (von Engelhardt et al., 2000). However, in this study individual rates of aggression did not correlate with androgen levels. It is interesting to note that T fluctuations do not always peak during the mating season. For instance, male muriqui monkeys (*Brachyteles arachnoides*) do not compete aggressively for mates, and fecal T concentrations do not increase during the mating season (Strier et al., 1999).

Although exceptions exist, the majority of research suggests that androgens do not necessarily increase aggression in nonhuman primates. For instance, in Japanese macaques seasonal increases in serum T and 5α-DHT levels were found to precede seasonal increases in aggression by 1–2 months, which was interpreted to mean that neither hormone affects aggression in a simple causal way (Rostal et al., 1986). Also, pharmacologically elevating T does not reliably increase rates of aggression. In rhesus monkeys, twice-weekly injections of human chorionic gonadotropin stimulated T production but had no consistent effect on rates of aggression (Gordon et al., 1979). Rather, increased circulating T levels were associated with an intensification of existing behavior, which did not disrupt group stability. On the other hand, Rejeski et al. (1990) showed evidence of a causal link between anabolic steroids and aggression in male long-tailed macaques (*M. fascicularis*). They found that injection of TP increased aggression in dominants, although it also increased submission in subordinates. When male marmosets (*Callithrix jacchus*) were castrated as neonates and tested as adults, they were found to display high rates of aggression with female partners and low rates with male partners (Dixson, 1993a). This pattern of aggression is opposite to that expressed by intact male marmosets. Further, the effects of neonatal castration on aggressive behavior were reversed by TP treatment in adulthood (Dixson, 1993b). Finally, several studies have found that castration of adult males has little effect on their aggressive behavior, suggesting that T is not required to maintain aggression in adults (Wilson and Vessey, 1968; Epple, 1978; Dixson, 1993c).

A common finding is that winning increases T, while losing decreases T. Among macaque males, animals that lost social conflicts showed a reduction in plasma T, especially if the defeat resulted in a fall in dominance rank (Rose et al., 1972; Bernstein et al., 1979). Similarly, on days when vervet (*Cercopithecus aethiops*) males fought, plasma T levels were higher in the winners than in the losers (Steklis et al., 1985). Also, several studies have shown that during periods of group formation plasma T concentrations increase in future dominants and decline in future subordinates, but that plasma T levels prior to group formation do not predict dominance rank (Rose et al., 1975; Mendoza et al., 1979; Keverne et al., 1982). In sum, these studies suggest that changes in T may be a consequence, rather than a cause, of aggression. In humans, physical aggression among males also seems unrelated to plasma T concentrations. Rather, T appears associated with the achievement and maintenance of high social status (Mazur and Booth, 1998). For instance, winning at tennis (Booth et al., 1989), chess (Mazur et al., 1992), or soccer (Neave and Wolfson, 2003) has been associated with transient increases in circulating T. Similarly, the rise in T during puberty has been related to nonaggressive symptoms of conduct disorder in boys with deviant peers, whereas it has been related to leadership in boys without deviant peers (Rowe et al., 2004).

Correlations between androgens and dominance rank appear to be mediated by the relationship between rank and aggression. This was demonstrated by the pioneering work of Sapolsky (1983; reviewed

in 1993) on olive baboons (*Papio anubis*). This work demonstrated that during periods of instability in the dominance hierarchy, dominant males were more aggressive and had greater plasma T concentrations than subordinates; in contrast, no correlations existed during stable periods. Since then, numerous studies have found that during stable periods, individual differences in rates of aggression and dominance rank do not correlate with T concentrations (e.g., Eaton and Resko, 1974; Steklis et al., 1986; Nieuwenhuijsen et al., 1987; van Schaik et al., 1991), whereas such correlations have been found during periods with unstable hierarchies or other social challenges (e.g., Wicklings and Dixson, 1992; Brockman et al., 1998). In chimpanzees, however, dominance rank has been correlated with afternoon (but not morning) urinary T concentrations during a period with a stable dominance hierarchy (Muller and Wrangham, 2004). A similar correlation has been found in chimpanzees by measuring fecal T during stable conditions (Muehlenbein et al., 2004). As the authors suggested, these correlations may be due to high-ranking males being more aggressive than low-ranking males regardless of hierarchy stability.

4.2 The Challenge Hypothesis

Recent studies on the neuroendocrinology of primate aggression have addressed the challenge hypothesis, which states that T levels are related to the degree of reproductive competition rather than mating activity (Wingfield et al., 1990; section 3.3). As in birds, T levels in primates appear more closely associated with competition than with reproduction. In primates, T facilitates spermatogenesis and sexual behavior but has little effect above minimum threshold levels (Weinbauer et al., 1988; Michael and Zumpe, 1993). In contrast, high rates of aggression have been associated with maximal plasma T levels (Rose et al., 1971). A similar relationship has been found between rates of aggression and T levels measured in CSF (Higley et al., 1996c). Only mild forms of aggression, however, correlate with increased T, whereas severe aggression (i.e., contact aggression such as biting) occurs throughout the year and tends to be independent of plasma and CSF T levels (Rose et al., 1978; Bernstein, 1983; Higley et al., 1996c). In Japanese macaques fecal T was found to correlate with noncontact aggression during the mating season, but no correlation was found with contact aggression (Barrett et al., 2002). Also, correlations between aggression and T levels predominately occur in competitive situations such as rank instability (Sapolsky, 1983; Higley et al., 1996c; Brockman et al., 2001) and mate competition (Cavigelli and Pereira, 2000; Barrett et al., 2002; Muller and Wrangham, 2004). Interspecies comparisons are also consistent with the challenge hypothesis. In multimale groups, where male reproductive competition is expected to be more frequent, breeding season concentrations of serum T are higher than those in unimale groups, where male challenges over mates occur more sporadically (Whitten, 2000).

Some primate studies have found mixed support for the challenge hypothesis, while others have suggested it needs a slight modification when applied to primates. In capuchin monkeys (*Cebus apella*), which show little direct mate competition among males, fecal T concentrations increased during a period of synchronized female sexual activity, but rates of male aggression did not correspond to the peak in T (Lynch et al., 2002). In male redfronted lemurs (*Eulemur fulvus*), fecal T concentrations tracked the increase in aggression observed during the mating season, but the highest T levels were recorded during the birth season (Ostner et al., 2002). These studies demonstrate that T levels can show a mating season peak without overt reproductive competition, and that T levels can be associated with social challenges outside a reproductive context. The risk of infanticide in some primate species may produce more predictable challenges, and may require males to elevate T and be prepared to aggressively defend infants against intruders during relatively well-defined periods of high risk (Ostner et al., 2002; Whitten et al., 2004). As previously mentioned, male chimpanzees respond to competition for estrous females with elevated urinary T (Muller and Wrangham, 2004), although urinary and fecal T concentrations remain positively correlated with dominance rank during periods of relative social stability (Muller and Wrangham, 2004; Muehlenbein et al., 2004). The fission–fusion society of chimpanzees, in which individuals regularly form small subgroups for foraging but occasionally join together in larger groups, creates unpredictable social challenges for males, and thus may require dominant males to maintain relatively high levels of circulating T to remain ready for potential confrontations with other males (Muller and Wrangham, 2004;

Muehlenbein et al., 2004). In addition, Muehlenbein et al. (2004) suggested that male chimpanzees may selectively lower T levels during infection to prevent the immunosuppressive effects of high circulating T.

4.3 Estrogens and Aggression

Although estrogens contribute to the expression of aggression in nonhuman primates, the available research is mixed. Some studies suggest that E_2 facilitates aggression in females; other studies suggest that estrogens inhibit aggression, and still others have found no effect. In rhesus macaques, females were found to display increased rates of noncontact forms of aggression (e.g., threats) during the middle of the menstrual cycle, when E2 peaks (Walker et al., 1983). Similarly, in ovariectomized rhesus, E_2 replacement has been shown to increase rates of noncontact aggression compared with nonhormone treated ovariectomized controls (Michael and Zumpe, 1993). In contrast, in long-tailed macaques ovariectomy without hormone replacement was shown to increase aggression, including biting, compared to sham-operated controls (Stavisky et al., 1999). A similar effect on aggression has been reported in a prosimian primate, the greater galago (*Galago crassicaudatus*). In this case, ovariectomy increased aggression and E_2 replacement decreased it (Dixson, 1978). Finally, a study on long-tailed macaques found no effect of ovariectomy on rates of aggression (Shively et al., 1986).

Clearly, the effect of estrogens on aggression in female nonhuman primates is equivocal. One possible reason for the variable results is the variety of social conditions used for behavioral testing such as male–female pairs, small same-sex groups, or large mixed-sex colonies. These different social conditions may also influence the type of aggressive behavior displayed such as contact and noncontact forms of aggression. Further, it should be noted that the reduction in E_2 produced by ovariectomy does not affect only aggression, but it can also decrease affiliation (Shively et al., 1986; Stavisky et al., 1999). Interestingly, recent data suggest that aggression in males may also be modulated by an estrogen-dependent pathway. In male long-tailed macaques, diets high in isoflavones, which are partial agonists of ERβ, have been associated with increased rates of aggression (Simon et al., 2004). Although aromatase inhibitors have been shown to decrease sexual behavior in male macaques (Zumpe et al., 1996), their effect of aggression has not been investigated. Indeed, the local metabolism of T into E_2 is an excellent area for future primate research.

Some studies also suggest that progesterone increases aggression in nonhuman primates. In vervet monkeys (*Cercopithecus aethiops*), aggression was shown to peak during the late luteal phase of the menstrual cycle, when progesterone levels are high (Rapkin et al., 1995). Also, medroxyprogesterone acetate (MPA), a synthetic progestin, has been shown to increase rates of aggression in female macaques (Linn and Steklis, 1990; Pazol et al., 2004). However, which steroid receptor mediates the effect of MPA is unclear.

4.4 Adrenal DHEA

Studies from rodents and birds suggest that androgens such as DHEA may regulate aggression in situations where aggression seems otherwise T-independent. Some studies in primates also suggest that aggressive behavior can be unrelated to fecal T levels (Lynch et al., 2002; Ostner et al., 2002). Although primate adrenal glands are known to secrete high levels of DHEA and DHEA-S (Rehman and Carr, 2004), the role of adrenal androgens in primate aggression is largely unknown. In some primate studies, measures of androgens may be confounded by adrenal DHEA and DHEA-S, causing difficulty in interpretation. For instance, Mohle et al. (2002) showed that in macaques metabolites of T and DHEA may cross-react with T antibodies in urine and fecal assays (Mohle et al., 2002). Thus, when a correlation is found between aggression and fecal or urinary androgens, it may be difficult to conclude with certainty the origin of the steroid. In addition, a lack of a correlation between aggression and excreted T does not necessarily mean that aggression is androgen-independent, as other androgens such as DHEA may regulate behavior.

One study has assessed circulating DHEA-S levels in a population of wild baboons (Sapolsky et al., 1993). They found that DHEA-S concentrations were high in both male and female baboons and showed

marked age-related decreases in both sexes; however, circulating levels of DHEA-S were not compared with aggression. Adrenal androgens appear to play a role in human aggression, as indicated by studies on conduct disorder, which is a term used to describe a collection of symptoms including aggression to people or animals, destruction of property, theft, and serious violations of rules. Prepubertal boys with conduct disorder were found to have higher levels of plasma DHEA-S, but not T, than normal control boys (van Goozen et al., 1998). Also, DHEA-S concentrations were correlated with the intensity of aggression as rated by parents and teachers. In another study, plasma DHEA-S concentrations were found to be higher in boys with conduct disorder than in boys with attention-deficit/hyperactivity disorder (ADHD) or normal controls (van Goozen et al., 2000). These studies suggest that adrenal androgens play an important role in the onset of aggression in adolescent boys. Adrenal androgens may also contribute to the regulation of aggression in human females. Adolescent and adult females with congenital adrenal hyperplasia, who were exposed to high levels of adrenal androgens in the prenatal and early postnatal periods, were found to have greater self-reported aggression ratings than were control females (Berenbaum and Resnick, 1997).

5 Conclusions

In rodents, birds, and primates, rates of aggression in males are often associated with circulating levels of T. This relationship has been extensively demonstrated in rodents, predominantly rats and mice, in which castration has been shown to reduce aggression and exogenous T treatment restores aggressive behavior (Edwards, 1969, 1970; Barfield, 1971). In birds, a positive correlation between plasma T and rates of aggression has been repeatedly documented during periods of social challenge, and particularly during mate competition (Wingfield et al., 1987). Likewise, recent data from primates has largely supported the challenge hypothesis (Cavigelli and Pereira, 2000; Barrett et al., 2002; Muller and Wrangham, 2004). These findings in primates build on previous work demonstrating a positive relationship between aggression and circulating T during periods of social instability (Sapolsky, 1983). It is important to remember, however, that in many cases, aggression is independent or even inversely related to circulating levels of T.

Seasonal aggression provides a valuable paradigm with which to study the neuroendocrine mechanisms of aggression. In many species (e.g., song sparrows, Siberian and Syrian hamsters, capuchin monkeys), high rates of aggression in males occur outside the period of high plasma T levels (Garrett and Campbell, 1980; Soma et al., 2000; Lynch et al., 2002, respectively). The neuroendocrine mechanisms regulating aggression under these conditions are poorly understood, but recent data provide some interesting possibilities. In birds, the metabolism of T to E_2 may mediate aggression during the mating season. During the nonmating season when circulating T is basal, steroid metabolism may still mediate aggression, albeit via a different endocrine mechanism (Soma et al., 2000). Despite low levels of circulating T, the androgen precursor DHEA remains elevated in blood, and conversion of DHEA to E_2 within the brain appears to play an important role in nonbreeding season aggression in some species (Soma et al., 2000; Soma and Wingfield, 2001).

In rodents, the aromatization of androgens also appears to regulate, at least in part, intermale aggression. Although seasonal changes in rodent aggression are less well-studied than in birds, recent data suggest that melatonin may mediate the increased aggression observed in hamsters during winter-like photoperiods (Jasnow et al., 2002; Demas et al., 2004). It seems likely that melatonin might regulate aggression under these conditions by affecting adrenal steroids such as cortisol and DHEA (Haus et al., 1996).

In nonhuman primates, little is known about the regulation of aggression when plasma T levels are low. In prepubertal boys, plasma DHEA-S levels have been associated with conduct disorder, and in particular with the aggressive symptoms of conduct disorder (van Goozen et al., 1998). Human and nonhuman primates secrete relatively high levels of DHEA and DHEA-S from the adrenal glands, but the role of DHEA in mediating nonhuman primate aggression has received little attention and is an exciting area for future research. An exclusive focus on T levels limits our understanding of the neuroendocrine regulation of aggression and strengthens the mistaken perception that aggression is simply a function of T. The idea that circulating T is not always associated with physical aggression, and instead is more often associated with

competition in specific situations, has been extended to humans (see Mazur and Booth, 1998). It seems likely that continued research on the role of steroid hormones in the regulation of aggression in animals will improve our understanding of aggression and violence in humans.

In addition to steroid hormones, a large number of peptides and neurotransmitters have been implicated in the regulation of aggression, including the neuropeptides oxytocin and vasopressin, as well as serotonin and nitric oxide among other factors. A brief discussion of only some of these factors could be presented here. It is important to keep in mind that, although each of these factors is typically studied in isolation, no single factor can sufficiently explain the wide range of aggressive behavior displayed by humans and nonhuman animals. Rather, a comprehensive examination of the multiple factors that regulate aggression and how these factors interact is needed to provide a complete understanding of the neuroendocrine regulation of aggression.

Acknowledgments

The authors would like to thank Dr. James Goodson, Dr. Michaela Hau, Melissa Scotti, and Amy Newman for helpful comments on the manuscript. This work was supported in part by NIH award NS10596, an HHMI Capstone award (G.E.D), NIH F32 award MH072085 (M.A.C), NIH award MH062641, and the STC program of the NSF (H.E.A) and NSERC, Canadian Institutes of Health Research, Michael Smith Foundation for Health Research, and Canada Foundation for Innovation (K.K.S).

References

Albers HE, Bamshad M. 1998. Role of vasopressin and oxytocin in the control of social behavior in Syrian hamsters (*Mesocricetus auratus*). Prog Brain Res 119: 395-408.

Albers HE, Huhman KL, Meisel RL. 2002. Hormonal basis of social conflict and communication. Hormones, brain and behavior. Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, editors. New York: Academic; pp. 393-433

Albert DJ, Jonik RH, Walsh ML. 1992. Hormone dependent aggression in male and female rats: Experimental, hormonal, and neural foundations. Neurosci Biobehav Rev 16: 177-192.

Arnold AP, Nottebohm F, Pfaff DW. 1976. Hormone concentrating cells in vocal control and other areas of brain of zebra finch (*Poephila guttata*). J Comp Neurol 165: 487-511.

Astheimer LB, Buttemer WA, Wingfield JC. 2000. Corticosterone treatment has no effect on reproductive hormones or aggressive behavior in free-living male tree sparrows, *Spizella arborea*. Horm Behav 37: 31-39.

Badura LL, Nunez AA. 1989. Photoperiodic modulation of sexual and aggressive behavior in female golden hamsters (*Mesocricetus auratus*): Role of the pineal gland. Horm Behav 23: 27-42.

Balthazart J, Baillien M, Charlier TD, Cornil CA, Ball GF. 2003. Multiple mechanisms control brain aromatase activity at the genomic and nongenomic level. J Steroid Biochem Molec Biol 86: 367-379.

Barfield RJ. 1971. Activation of sexual and aggressive behavior by androgen implanted into male ring dove brain. Endocrinology 89: 1470-1477.

Barfield RJ, Ronay G, Pfaff DW. 1978. Autoradiographic localization of androgen-concentrating cells in brain of male domestic fowl. Neuroendocrinology 26: 297-311.

Barrett GM, Shimizu K, Bardi M, Asaba S, Mori A. 2002. Endocrine correlates of rank, reproduction, and female-directed aggression in male Japanese macaques (*Macaca fuscata*). Horm Behav 42: 85-96.

Baulieu EE. 1981. Steroid hormone regulation of the brain. Fuxe K, Gustafsson JA, editors. Oxford: Pergamon Press; pp. 3-14.

Baulieu EE. 1991. Neurosteroids: A new function in the brain. Biol Cell 71: 3-10.

Bayart F, Spetz JP, Cittone L, Haug M. 1989. The role of gender and hormonal state on aggression during encounters between residential and intruder mice. Med Sci Res 17: 517-519.

Beeman EA. 1947. The effect of male hormone on aggressive behavior in mice. Physiol Zool 20: 373-404.

Beletsky LD, Orians GH, Wingfield JC. 1990. Effects of exogenous androgen and antiandrogen on territorial and nonterritorial red-winged blackbirds. Ethology 85: 58-72.

Berenbaum SA, Resnick SM. 1997. Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. Psychoneuroendocrinology 22: 505-15.

Bernard DJ, Bentley GE, Balthazart J, Turek FW, Ball GF. 1999. Androgen receptor, estrogen receptor α, and estrogen receptor β show distinct patterns of expression in forebrain song control nuclei of European starlings. Endocrinology 140: 4633-4643.

Bernstein I, Williams L, Ramsay M. 1983. The expression of aggression in Old World monkeys. Int J Primatol 4: 113-125.

Berthold AA. 1849. Transplantation of testes. Trans. By DP Quiring. Bull History Med, 1944 16: 399-401.

Bester-Meredith JK, Marler CA. 2001. Vasopressin and aggression in cross-fostered California mice (*Peromyscus californicus*) and white-footed mice (*Peromyscus leucopus*). Horm Behav 40: 51-64.

Bevan JM, Bevan W, Williams BF. 1958. Spontaneous aggressiveness in young castrate C3H mice treated with three dose levels of testosterone. Physiol Zool 31: 284-288.

Bevan WD, Daves WF, Levy FW. 1960. The relation of castration androgen therapy, and pre-test fighting experience to competitive aggression in male C57BL/10 mice. Anim Behav 8: 6-12.

Blanchard DC, Blanchard RJ. 1988. Ethoexperimental approaches to the biology of emotion. Annu Rev Psychol 39: 43-68.

Blanchard DC, Spencer RL, Weiss SM, Blanchard RJ, McEwen B, et al. 1995. Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. Psychoneuroendocrinology 20: 117-134.

Booth A, Shelley G, Mazur A, Tharp G, Kittok R. 1989. Testosterone and winning and losing in human competition. Horm Behav 23: 556-571.

Bottriech A, Zuir I, Hammel I, Terkel J. 2001. Non-involvement of testosterone in aggressive defense behavior in the male blind mole rat *Spalax ehrenbergi*. Agg Behav 27: 64-72.

Brain PF. 1972. Mammalian behavior and the adrenal cortex. A review. Behav Biol 7: 453-477.

Brain PF. 1975. What does individual housing mean to a mouse? Life Sci 16: 187-200.

Brain PF, Poole AE. 1974. Some studies on the use of 'standard opponents' in intermale aggression testing in TT albino mice. Behaviour 50: 100-110.

Brain PF, Haug M. 1992. Hormonal and neurochemical correlates of various forms of animal "aggression". Psychoneuroendocrinology 17: 537-551.

Brenowitz EA. 1997. Comparative approaches to the avian song system. J Neurobiol 33: 517-531.

Brenowitz EA, Margoliash D, Nordeen KW. 1997. An introduction to birdsong and the avian song system. J Neurobiol 33: 495-500.

Brockman DK, Whitten PL, Richard AF, Schneider A. 1998. Reproduction in free-ranging male Propithecus verreauzi: The hormonal correlates of mating and aggression. Am J Phys Anthropol 105: 137-151.

Brockman DK, Whitten PL, Richard AF, Benander B. 2001. Birth season testosterone levels in male Verreaux's sifaka, Propithecus verreauxi: Insights into socio-demographic factors mediating seasonal testicular function. Behav Ecol Sociobiol 49: 117-127.

Bronson FH, Desjardins C. 1969. Aggressive behavior and seminal vesicle function in mice: Differential sensitivity to androgen given neonatally. Endocrinology 85: 971-974.

Bronson FH, Desjardins C. 1970. Neonatal androgen administration and adult aggressiveness in female mice. Gen Comp Endocrinol 15: 320-325.

Buck CL, Barnes BM. 2003. Androgen in free-living arctic ground squirrels: Seasonal changes and influence of staged male-male aggressive encounters. Horm Behav 43: 318-326.

Caldwell HK, Albers HE. 2004. Effect of photoperiod on vasopressin-induced aggression in Syrian hamsters. Horm Behav 46: 444-449.

Caldwell GS, Glickman SE, Smith ER. 1984. Seasonal aggression independent of seasonal testosterone in wood rats. Proc Natl Acad Sci USA 81: 5255-5257.

Canoine V, Gwinner E. 2002. Seasonal differences in the hormonal control of territorial aggression in free-living European stonechats. Horm Behav 41: 1-8.

Casto JM, Nolan V, Ketterson ED. 2001. Steroid hormones and immune function: Experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). Am Nat 157: 408-420.

Cavigelli SA, Pereira ME. 2000. Mating season aggression and fecal testosterone levels in male ring-tailed lemurs (*Lemur catta*). Horm Behav 37: 246-255.

Chapais B. 1992. The role of alliances in the social inheritance of rank among female primates. Coalitions and alliances in humans and other animals. Harcourt AH, de Waal FBM, editors. Oxford: Oxford University Press; pp. 29-60.

Chiavegatto S, Nelson RJ. 2003. Interaction of nitric oxide and serotonin in aggressive behavior. Horm Behav 44: 233-241.

Christianson T, Wallen K, Brown B, Glickman SE. 1972. Effects of castration, blindness, and anosmia on social activity in the male Mongolian gerbil (*Meriones unguiculatus*). Physiol Behav 10: 989-994.

Ciacco LA, Lisk RD, Rueter LA. 1979. Prelordotic behavior in the hamster: A hormonally modulated transition from aggression to sexual receptivity. J Comp Physiol Psychol 93: 771-780.

Cooper MA, Huhman KL. 2003. Corticotropin-releasing hormone, and not glucocorticoids, modulated the acquisition

of conditioned defeat in male Syrian hamsters. San Diego, CA: Society for Neuroscience.

Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu EE. 1981. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci 78: 4704-4707.

Davis DE. 1957. Aggressive behavior in castrated starlings. Science 130: 253.

Delville Y, Hendrick JC, Sulon J, Balthazart J. 1984. Testosterone metabolism and testosterone-dependent characteristics in Japanese quail. Physiol Behav 33: 817-823.

Demas GE, Moffatt CA, Drazen DL, Nelson RJ. 1999b. Castration does not inhibit aggressive behavior in adult male prairie voles. Physiol Behav 6: 59-61.

Demas GE, Polacek KM, Durazzo A, Jasnow AM. 2004. Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*). Horm Behav 46: 582-591.

Dewsbury DA. 1991. Individual attributes generate contrasting degrees of sociality in voles. Social systems and population cycles in voles. Tamarkin RH, Ostfeld RS, Pugh SR, Bujalska G, editors. Boston: Birkhäuser Verlag; pp. 1-9.

DeLeon KR, Grimes JM, Melloni RH Jr. 2002. Repeated anabolic-androgenic steroid treatment during adolescence increases vasopressin V(1a) receptor binding in Syrian hamsters: Correlation with offensive aggression. Horm Behav 42: 182-191.

Delville Y, David JT, Taravosh-Lahn K, Wommack JC. 2003. Stress and the development of agonistic behavior in golden hamsters. Horm Behav 44: 263-270.

DeVoogd TJ, Krebs JR, Healy SD, Purvis A. 1993. Relations between song repertoire size and the volume of brain nuclei related to song: Comparative evolutionary analyses amongst oscine birds. Proc Roy Soc London B 254: 75-82.

Dixson AF. 1978. Effects of ovariectomy and estradiol replacement therapy upon the sexual and aggressive behavior of the greater galago (*Galago crassicaudatus crassicaudatus*). Horm Behav 10: 61-70.

Dixson AF. 1993a. Observations on effects of neonatal castration upon sexual and aggressive behavior in the male common marmoset (*Callithrix jacchus*). Am J Primatol 31: 1-10.

Dixson AF. 1993b. Effects of testosterone propionate upon the sexual and aggressive behavior of adult male marmosets (*Callithrix jacchus*) castrated as neonates. Horm Behav 27: 216-230.

Dixson AF. 1993c. Sexual and aggressive behavior of adult male marmosets (*Callithrix jacchus*) castrated neonatally, prepubertally, or in adulthood. Physiol Behav 54: 301-307.

Dufty AM. 1989. Testosterone and survival: A cost of aggressiveness. Horm Behav 23: 185-193.

Eaton GG, Resko JA. 1974. Plasma testosterone and male dominance in a Japanese macaque (*Macaca fuscata*) troop compared with repeated measures of testosterone in laboratory males. Horm Behav 5: 251-259.

Edwards DA. 1968. Mice: Fighting by neonatally androgenized females. Science 161: 1027-1028.

Edwards DA. 1969. Early androgen stimulation and aggressive behavior in male and female mice. Physiol Behav 4: 333-338.

Edwards DA. 1970. Post-neonatal androgenization and adult aggressive behavior in female mice. Physiol Behav 5: 465-446.

Epple G. 1978. Lack of effects of castration on scent marking, displays, and aggression in a South American primate (*Saguinus fuscicollis*). Horm Behav 11: 139-150.

Farner DS, Wingfield JC. 1980. Reproductive endocrinology of birds. Annu Rev Physiol 42: 457-472.

Ferguson JN, Young LJ, Insel TR. 2002. The neuroendocrine basis of social recognition. Front Neuroendocrinol 23: 200-224.

Ferree ED, Wikelski MC, Anderson DJ. 2004. Hormonal correlates of siblicide in Nazca boobies: Support for the challenge hypothesis. Horm Behav 46: 655-662.

Ferris CF, Albers HE, Wesolowski SM, Goldman BD, Luman SE. 1984. Vasopressin injected into the hypothalamus triggers a stereotypic behavior in golden hamsters. Science 224: 521-523.

Ferris CF, Meenan DM, Axelson JF, Albers HE. 1986. A vasopressin antagonist can reverse dominant/subordinate behavior in hamsters. Physiol Behav 38: 135-138.

Ferris CF, Delville Y. 1994. Vasopressin and serotonin interactions in the control of agonistic behavior. Psychoneuroendocrinology 19: 593-601.

Ferris CF, Delville Y, Brewer JA, Mansour K, Yules B, Melloni RH Jr. 1996. Vasopressin and developmental onset of flank marking behavior in golden hamsters. J Neurobiol 30: 192-204.

Ferris CF, Melloni RH, Koppel G, Perry KW, Fuller RW, et al. 1997. Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in Syrian hamsters. J Neurosci 17: 4331-4340.

Fleming AS, Phillips A, Rydall A, Levesque L. 1988. Effects of photoperiod, the pineal gland and the gonads on agonistic behavior in female golden hamsters (*Mesocricetus auratus*). Physiol Behav 44: 227-234.

Fusani L, Van't Hof T, Hutchison JB, Gahr M. 2000. Seasonal expression of androgen receptors, estrogen receptors, and aromatase in the canary brain in relation to circulating androgens and estrogens. J Neurobiol 43: 254-268.

Gahr M, Guttinger HR, Kroodsma DE. 1993. Estrogen receptors in the avian brain: Survey reveals general distribution

and forebrain areas unique to songbirds. J Comp Neurol 327: 112-122.

Garrett JW, Campbell CS. 1980. Changes in social behavior of the male golden hamster accompanying photoperiodic changes in reproduction. Horm Behav 14: 303-319.

Glick BB. 1979. Testicular size, testosterone level, and body weight in male *Macaca radiata*. Folia Primatol 32: 268-289.

Goldman L, Swanson HH. 1975. Developmental changes in pre-adult behavior in confined colonies of golden hamsters. Dev Psychobiol 8: 137-150.

Goldman BD. 2001. Mammalian photoperiodic system: Formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16: 283-301.

Goodson JL. 1998a. Territorial aggression and dawn song are modulated by septal vasotocin and vasoactive intestinal polypeptide in male field sparrows (*Spizella pusilla*). Horm Behav 34: 67-77.

Goodson JL. 1998b. Vasotocin and vasoactive intestinal polypeptide modulate aggression in a territorial songbird, the violet-eared waxbill (Estrildidae: Uraeginthus granatina). Gen Comp Endocrinol 111: 233-244.

Goodson JL, Adkins-Regan E. 1999. Effect of intraseptal vasotocin and vasoactive intestinal polypeptide infusions on courtship song and aggression in the male zebra finch (*Taeniopygia guttata*). J Neuroendocrinol 11: 19-25.

Goodson JL, Bass AH. 2001. Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. Brain Res Rev 35: 246-265.

Goodson JL, Evans AK. 2004. Neural responses to territorial challenge and nonsocial stress in male song sparrows: Segregation, integration, and modulation by a vasopressin V1 antagonist. Horm Behav 46: 371-381.

Goodson JL, Evans AK, Lindberg L, Allen CD. 2005. Neuroevolutionary patterning of sociality. Proc Roy Soc B 272: 227-235.

Goodson JL, Saldanha CJ, Hahn TP, Soma KK. 2005. Recent advances in behavioral neuroendocrinology: Insights from studies on birds. Horm Behav 48(4): 461-473.

Gordon TP, Rose RM, Bernstein IS. 1976. Seasonal rhythm in plasma testosterone levels in the rhesus monkey (*Macaca mulatta*): A three year study. Horm Behav 7: 229-243.

Gordon TP, Bernstein IS, Rose RM. 1978. Social and seasonal influences on testosterone secretion in the male rhesus monkey. Physiol Behav 21: 623-627.

Gordon TP, Rose RM, Grady CL, Bernstein IS. 1979. Effects of increased testosterone secretion on the behavior of adult male rhesus living in a social group. Folia Primatol 32: 149-160.

Gottreich A, Zuri I, Hammel I, Terkel J. 2001. Noninvolvement of testosterone in aggressive defense behavior in the male blind mole rat Spalax ehrenbergi. Agg Behav 27: 64-72.

Goymann W, Moore IT, Scheuerlein A, Hirschenhauser K, Grafen A, et al. 2004. Testosterone in tropical birds: Effects of environmental and social factors. Am Nat 164: 327-334.

Gwinner E, Rodl T, Schwabl H. 1994. Pair territoriality of wintering stonechats: Behavior, function and hormones. Behav Ecol Sociobiol 34: 321-327.

Haller J, Kruk MR. 2003. Neuroendocrine stress responses and aggression. Mattson MP, editor. Neurobiology of Aggression. NJ: Humana Press; Totawa, pp. 93-118.

Harding CF, Follett BK. 1979. Hormone changes triggered by aggression in a natural population of blackbirds. Science 203: 918-920.

Harding CF, Walters MJ, Collado D, Sheridan K. 1988. Hormonal specificity and activation of social behavior in male red-winged blackbirds. Horm Behav 22: 402-418.

Harding CF, Walters MJ, Parsons B. 1984. Androgen receptor levels in hypothalamic and vocal control nuclei in the male zebra finch. Brain Res 106: 333-339.

Hau M, Wikelski M, Soma KK, Wingfield JC. 2000. Testosterone and year-round territorial aggression in a tropical bird. Gen Comp Endocrinol 117: 20-33.

Hau M, Stoddard ST, Soma KK. 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Horm Behav 45: 40-49.

Haug M, Johnson F, Brain PF. 1992. Biological correlates of attack on lactating intruders by female mice: A topical review. Bjorkvist K, Niemala P. (eds). Of mice and women In Aspects of female Aggression Academic Press: San Diego, pp 381-393.

Haus E, Nicolau GY, Ghinea E, Dumitriu L, Petrescu E, et al. 1996. Stimulation of the secretion of dehydroepiandrosterone by melatonin in mouse adrenals in vitro. Life Sci 58: PL263-PL267.

Hegner RE, Wingfield JC. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. Auk 104: 462-469.

Herndon JG, Bein ML, Nordmeyer DL, Turner JJ. 1996. Seasonal testicular function in male rhesus monkeys. Horm Behav 30: 266-271.

Higley JD, Mehlman PT, Poland RE, Taub DM, Vickers J, et al. 1996c. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol Psychiat 40: 1067-1082.

Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, Israel JE, Jasnow AM. 2003. Conditioned defeat in male and female Syrian hamsters. Horm Behav 44: 293-299.

Hunt K, Wingfield JC, Astheimer LB, Buttemer WA, Hahn TP. 1995. Temporal patterns of territorial behavior and circulating testosterone in the lapland longspur and other Arctic passerines. Am Zool 35: 274-284.

Hunt KE, Hahn TP, Wingfield JC. 1997. Testosterone implants increase song but not aggression in male Lapland longspurs. Anim Behav 54: 1177-1192.

Jasnow AM, Huhman KL, Bartness TJ, Demas GE. 2000. Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). Horm Behav 38: 102-110.

Jasnow AM, Huhman KL, Bartness TJ, Demas GE. 2002. Short days and exogenous melatonin increase aggression in male Syrian hamsters (*Mesocricetus auratus*). Horm Behav 42: 13-20.

Ketterson ED. 1979. Aggressive behavior in wintering dark-eyed juncos: Determinants of dominance and their possible relation to geographic variation in sex ratio. Wilson Bulletin 91: 371-383.

Ketterson ED, Nolan V. 1999. Adaptation, exaptation, and constraint: A hormonal perspective. Am Nat 154: S4-S25.

Ketterson ED, Nolan V, Cawthorn MJ, Parker PG, Ziegenfus C. 1996. Phenotypic engineering: Using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138: 70-86.

Ketterson ED, Nolan V, Wolf L, Ziegenfus C. 1992. Testosterone and avian life histories: Effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). Am Nat 140: 980-999.

Ketterson ED, Nolan V, Wolf L, Ziegenfus C, Dufty AM, et al. 1991. Testosterone and avian life histories: The effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. Horm Behav 25: 489-503.

Keverne EB, Meller RE, Eberhart A. 1982. Dominance and subordination: Concepts and physiological states. Advanced views in primate biology. Chiarelli AB, Corruccini RS, editors. New York: Springer-Verlag; pp. 81-94.

Kim YH, Perlman WR, Arnold AP. 2004. Expression of androgen receptor mRNA in zebra finch song system: Developmental regulation by estrogen. J Comp Neurol 469: 535-547.

Konishi M, Emlen ST, Ricklefs RE, Wingfield JC. 1989. Contributions of bird studies to biology. Science 246: 465-472.

Kriner E, Schwabl H. 1991. Control of winter song and territorial aggression of female robins (*Erithacus rubecula*) by testosterone. Ethology 87: 37-44.

Kroboth PD, Salek FS, Pittenger AL, Fabian TJ, Frye RF. 1999. DHEA and DHEA-S: A review. J Clin Pharmacol 39: 327-348.

Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez JL, Candas B. 1998. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: Its role during aging. Steroids. 63: 322-328.

Labrie F, Luu-The V, Labrie C, Simard J. 2001. DHEA and its transformation into androgens and estrogens in peripheral target tissues: Intracrinology. Front Neuroendocrinol 22: 185-212.

Levin RN, Wingfield JC. 1992. The hormonal control of territorial aggression in tropical birds. Ornis Scand 23: 284-291.

Linn FS, Steklis HD. 1990. The effects of depo-medroxyprogesterone acetate (DMPA) on copulation-related and agonistic behaviors in an island colony of stumptail macaques (*Macaca arctoides*). Physiol Behav 47: 403-408.

Lohiya NK, Sharma RS, Manivannan B, Kumar TCA. 1998. Reproductive exocrine and endocrine profiles and their seasonality in male langur monkeys (*Presbytis entellus entellus*). J Med Primatol 27: 15-20.

Logan CA, Carlin CA. 1991. Testosterone stimulates reproductive behavior during autumn in mockingbirds (*Mimus polyglottos*). Horm Behav 25: 229-241.

Logan CA, Wingfield JC. 1990. Autumnal territorial aggression is independent of plasma testosterone in mockingbirds. Horm Behav 24: 568-581.

Lu SF, Mo Q, Hum S, Garippam C, Simon NG. 2003. Dehydroepiandrosterone upregulates neural androgen receptor level and transcriptional activity. J Neurobiol 57: 163-171.

Lynch JW, Ziegler TE, Strier KB. 2002. Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys, *Cebus apella nigritus*. Horm Behav 41: 275-287.

Marques DM, Valenstein ES. 1977. Individual differences in aggressiveness of female hamsters: Response to intact and castrated males and to females. Anim Behav 25: 131-139.

Martin LB, Pless M, Svoboda J, Wikelski M. 2004. Immune activity in temperate and tropical house sparrows: A common-garden experiment. Ecology 85: 2323-2331.

Mason WA. 1993. The nature of social conflict: A psychoethological perspective. Mason WA, Mendoza SP (eds). Primate Social Conflict. Albany: SUNY Press; pp. 13-47.

Matsumoto T, Honda S, Harada N. 2003. Neurological effects of aromatase deficiency in the mouse. J Steroid Biochem Mol Biol 86: 357-365.

Mathewson SF. 1961. Gonadotrophic hormones affect aggressive behavior in starlings. Science 134: 1522-1523.

Mazur A, Booth A. 1998. Testosterone and dominance in men. Behav Brain Sci 21: 353-397.

Mazur A, Booth A, Dabbs JM. 1992. Testosterone and chess competition. Soc Psychol Q 55: 70-77.

Mellon SH, Vaudry H. 2001. Biosynthesis of neurosteroids and regulation of their synthesis. Int Rev Neurobiol 46: 33-78.

Mendoza SP, Coe CL, Lowe EL, Levine S. 1979. The physiological response to group formation in adult male squirrel monkeys. Psychoneuroendocrinology 3: 221-229.

Michael RP, Zumpe D. 1993. A review of hormonal factors influencing the sexual and aggressive behavior of macaques. Am J Primatol 30: 213-241.

Miczek KA, DeBold JF, van Erp AM. 1994. Neuropharmacological characteristics of individual differences in alcohol effects on aggression in rodents and primates. Behav Pharmacol 5: 407-421.

Mohle U, Heistermann M, Palme R, Hodges JK. 2002. Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates. Gen Comp Endocrinol 129: 135-145.

Moss R, Parr R, Lambin X. 1994. Effects of testosterone on breeding density, breeding success and survival of red grouse. Proc Roy Soc London B 258: 175-180.

Mougeot F, Dawson A, Redpath SM, Leckie F. 2005. Testosterone and autumn territorial behavior in male red grouse, *Lagopus lagopus scoticus*. Horm Behav 47: 576-584.

Moyer KE. The Physiology of Hostility. Chicago: Markham; 1971.

Muehlenbein MP, Watts DP, Whitten PL. 2004. Dominance rank and fecal testosterone levels in adult male chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda. Am J Primatol 64: 71-82.

Muller MN, Wrangham RW. 2004. Dominance, aggression and testosterone in wild chimpanzees: A test of the 'challenge hypothesis'. Anim Behav 67: 113-123.

Neave N, Wolfson S. 2003. Testosterone, territoriality, and the 'home advantage'. Physiol Behav 78: 269-275.

Nelson, RJ. 1990. Photoperiodic responsiveness in house mice. Physiol Behav 48: 403-408.

Nicolas LB, Pinoteau W, Papot S, Routierm S, Guillaumetm G, et al. 2001. Aggressive behavior induced by the steroid sulfatase inhibitor COUMATE and by DHEAS in CBA/H mice. Brain Res 922: 216-222.

Nieuwenhuijsen K, de Neef KJ, van der Werff ten Bosch JJSAK. 1987. Testosterone, testis size, seasonality, and behavior in group-living stumptail macaques (*Macaca arctoides*). Horm Behav 21: 153-169.

Nomura M, Durbak L, Chan J, Smithies O, Gustafsson JA, Korach KS, Pfaff DW, Ogawa S. 2002. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor beta knockout (betaERKO) male mice. Horm Behav 41: 288-296.

Nottebohm F, Stokes TM, Leonard CM. 1976. Central control of song in the canary, *Serinus canarius*. J Comp Neurol 165: 457-486.

Ogawa S, Lubahnm DB, Korach KS, Pfaff DW. 1997. Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci USA 94: 1476-1481.

Ogawa S, Washburn TF, Taylor J, Lubahn DB, Korach KS, et al. 1998. Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. Endocrinology 139: 5058-5069.

Ogawa S, Chanm J, Chesterm AE, Gustafssonm JA, Korach KS, et al. 1999. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc Natl Acad Sci USA 96: 12887-12892.

Ogawa S. 2003. Differential roles of estrogen receptor alpha and beta in the regulation of neuroendocrine functions and socio-sexual behaviors. J Nippon Med Sch 70: 530.

Ostner T, Kappeler PM, Heistermann M. 2002. Seasonal variation and social correlates of androgen excretion in male redfronted lemurs (*Eulemur fulvus rufus*). Behav Ecol Sociobiol 52: 485-495.

Owen-Ashley NT, Hasselquist D, Wingfield JC. 2004. Androgens and the immunocompetence handicap hypothesis: Unraveling direct and indirect pathways of immunosuppression in song sparrows. Am Nat 164: 490-505.

Paterson AT, Vickers C. 1981. Melatonin and the adrenal cortex: Relationship to territorial aggression in mice. Physiol Behav 27: 983-987.

Pazol K, Wilson ME, Wallen K. 2004. Medroxyprogesterone acetate antagonizes the effects of estrogen treatment on social and sexual behavior in female macaques. J Clin Endocrinol Metabol 89: 2998-3006.

Pellis SM, Pellis VC. 1998. Play fighting of rats in comparative perspective: A schema for neurobehavioral analyses. Neurosci Biobehav Rev 23: 87-101.

Perché F, Young J, Robel P, Simon NG, Haug M. 2000. Prenatal testosterone treatment potentates the aggression-suppressive effect of the neurosteroid dehydroandrosterone in female mice. Agg Behav 27: 130-138.

Perlman WR, Arnold AP. 2003. Expression of estrogen receptor and aromatase mRNAs in embryonic and posthatch zebra finch brain. J Neurobiol 55: 204-219.

Perret M. 1992. Environmental and social determinants of sexual function in the male lesser mouse lemur (*Microcebus murinus*). Folia Primatol 59: 1-25.

Phillips RE, Barfield RJ. 1977. Effects of testosterone implants in midbrain vocal areas of capons. Brain Res 122: 378-381.

Phoenix CH, Goy RW, Gerall AA, Young WC. 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65: 369-382.

Pieper DR, Lobocki CA. 2000. Characterization of serum dehydroepiandrosterone secretion in golden hamsters. Proc Soc Exp Biol Med 224: 278-284.

Pinna G, Costa E, Guidotti A. 2005. Changes in brain testosterone and allopregnanolone biosynthesis elicit aggressive behavior. Proc Natl Acad Sci USA. 102: 2135-2140.

Pinxten R, De Ridder E, Balthazart J, Berghman L, Eens M. 2000. The effect of castration on aggression in the

nonbreeding season is age-dependent in male European starlings. Behaviour 137: 647-661.

Pinxten R, De Ridder E, Balthazart J, Eens M. 2002. Context-dependent effects of castration and testosterone treatment on song in male European starlings. Horm Behav 42: 307-318.

Pinxten R, De Ridder E, De Cock M, Eens M. 2003. Castration does not decrease nonreproductive aggression in yearling male European starlings (*Sturnus vulgaris*). Horm Behav 43: 394-401.

Potegal M, Ferris CF. 1990. Intraspecific aggression in male hamsters is inhibited by intrahypothalamic VP receptor antagonist. Aggressive Behav 15: 311-320.

Rapkin AJ, Pollack DB, Raleigh MJ, Stone B, McGuire MT. 1995. Menstrual cycle and social behavior in vervet monkeys. Psychoneuroendocrinology 20: 289-297.

Rehman KS, Carr BR. 2004. Sex differences in adrenal androgens. Semin Reprod Med 22: 349-360.

Rejeski WJ, Gregg E, Kaplan JR, Manuck SB. 1990. Anabolic androgenic steroids: Effects on social behavior and baseline heart rate. Health Psychol 9: 774-791.

Ros AFH, Dieleman SJ, Groothuis TGG. 2002. Social stimuli, testosterone, and aggression in gull chicks: Support for the challenge hypothesis. Horm Behav 41: 334-342.

Rose RM, Bernstein IS, Gordon TP. 1975. Consequences of social conflict on plasma testosterone levels in rhesus monkeys. Psychosom Med 37: 50-61.

Rose RM, Holaday JW, Bernstein IS. 1971. Plasma testosterone, dominance rank and aggressive behavior in male rhesus monkeys. Nature 231: 366-368.

Rose RM, Gordon TP, Bernstein IS. 1972. Plasma testosterone levels in the male rhesus: Influences of sexual and social stimuli. Science 178: 643-645.

Rose RM, Gordon TP, Bernstein IS. 1978. Diurnal variation in plasma testosterone and cortisol in rhesus monkeys living in social groups. J Endocrinol 76: 67-74.

Rostal DC, Glick BB, Eaton GC, Resko JA. 1986. Seasonality of adult male Japanese macaques (*Macaca fuscata*): Androgens and behavior in a confined troop. Horm Behav 20: 452-462.

Rowe R, Maughan B, Worthman CM, Costello EJ, Angold A. 2004. Testosterone, antisocial behavior, and social dominance in boys: Pubertal development and biosocial interaction. Biol Psychiat 55: 546-552.

Ruiz de Elvira MC, Herndon JG, Wilson ME. 1982. Influence of estrogen-treated females on sexual behavior and male testosterone levels of a social group of rhesus monkeys during the nonbreeding season. Biol Reprod 26: 825-834.

Saldanha CJ, Coomaralingam L. 2005. Overlap and co-expression of estrogen synthetic and responsive neurons in the songbird brain: A double-label immunocytochemical study. Gen Comp Endocrinol 141: 66-75.

Sapolsky RM. 1983. Endocrine aspects of social instability in the olive baboon (*Papio anubis*). Am J Primatol 5: 365-379.

Sapolsky RM, Vogelman JH, Orentreich N, Altmann J. 1993. Senescent decline in serum dehydroepiandrosterone sulfate concentrations in a population of wild baboons. J Gerontol 48: B196-B200.

Schiml PA, Mendoza SP, Saltzman W, Lyons DM, Mason WA. 1996. Seasonality in squirrel monkeys (*Saimiri sciureus*), social facilitation by females. Physiol Behav 60: 1105-1113.

Schlegel ML, Spetz JF, Robel P, Haug M. 1985. Studies on the effects of dehydroepiandrosterone and its metabolites on attack by castrated mice on lactating intruders. Physiol Behav 34: 867-870.

Schleussner G, Gwinner E. 1989. Photoperiodic time measurement during the termination of photorefractoriness in the starling (*Sturnus vulgaris L.*). Gen Comp Endocrinol 75: 54-61.

Schlinger BA. 1997. Sex steroids and their actions on the birdsong system. J Neurobiology 33: 619-631.

Schlinger BA, Arnold AP. 1992. Circulating estrogens in a male songbird originate in the brain. Proc Natl Acad Sci 89: 7650-7653.

Schlinger BA, Callard GV. 1987. A comparison of aromatase, 5α-, and 5β-reductase activities in the brain and pituitary of male and female quail. J Exp Zool 242: 171-180.

Schlinger BA, Callard GV. 1989a. Aromatase activity in quail brain: Correlation with aggressiveness. Endocrinology 124: 437-443.

Schlinger BA, Callard GV. 1989b. Estrogen receptors in quail brain: A functional relationship to aromatase and aggressiveness. Biol Reprod 40: 268-275.

Schlinger BA, Callard GV. 1990a. Aggressive behavior in birds: An experimental model for studies of brain-steroid interactions. Comp Biochem Physiol A 97: 307-316.

Schlinger BA, Callard GV. 1990b. Aromatization mediates aggressive behavior in quail. Gen Comp Endocrinol 79: 39-53.

Schwabl H. 1992. Winter and breeding territorial behavior and levels of reproductive hormones of migratory European robins. Ornis Scand 23: 271-276.

Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci 90: 11446-11450.

Schwabl H, Kriner E. 1991. Territorial aggression and song of male European robins (*Erithacus rubecula*) in autumn and spring-effects of antiandrogen treatment. Horm Behav 25: 180-194.

Searcy WA, Wingfield JC. 1980. The effects of androgen and antiandrogen on dominance and aggressiveness in male red-winged blackbirds. Horm Behav 14: 126-135.

Setchell JM, Dixson AF. 2001. Circannual changes in the secondary sexual adornments of semifree-ranging male

and female mandrills (*Mandrillus sphinx*). Am J Primatol 53: 109-121.

Shively CA, Kaplan JR, Adams MR. 1986. Effects of ovariectomy, social instability and social status on female *Macaca fascicularis* social behavior. Physiol Behav 36: 1147-1153.

Sigg EB. 1969. Relationship of aggressive behavior to adrenal and gonadal function in male mice. Aggressive behavior (monograph). Garattini S, Sigg EB, editors. Amsterdam: Excerpta Medica.

Silverin B, Baillien M, Balthazart J. 2004. Territorial aggression, circulating levels of testosterone, and brain aromatase activity in free-living pied flycatchers. Horm Behav 45: 225-234.

Silverin B, Viebke PA, Westin J. 1984. Plasma levels of luteinizing hormone and steroid hormones in free-living winter groups of willow tits (*Parus montanus*). Horm Behav 18: 367-379.

Simon NG. 2002. Hormonal processes in the development and expression of aggressive behavior. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, (eds). Hormones, Brain and behavior, vol. 1., New York: Academic Press. pp. 339-392.

Simon NG, Kaplan JR, Hu S, Register TC, Adams MR. 2004. Increased aggressive behavior and decreased affiliative behavior in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones. Horm Behav 45: 278-284.

Scott JP, Fredericson E. 1951. The causes of fighting in mice and rats. Physiol Zool 24: 273.

Smulders TV, Casto JM, Nolan V Jr, Ketterson ED, DeVoogd TJ. 2000. Effects of captivity and testosterone on the volumes of four brain regions in the dark-eyed junco (*Junco hyemalis*). J Neurobiol 43: 244-253.

Soma KK. 2004. Steroid regulation of territorial behavior in wild songbirds. Horm Behav 46: 135.

Soma KK, Alday NA, Hau M, Schlinger BA. 2004. Dehydroepiandrosterone metabolism by 3β-hydroxysteroid dehydrogenase/δ5-δ4 isomerase in adult zebra finch brain: Sex difference and rapid effect of stress. Endocrinology 145: 1668-1677.

Soma KK, Alday NA, BA. Schlinger 2002. 3β-HSD and aromatase in songbird brain: DHEA metabolism, aggression, and song. Society for Neuroscience Abstracts.

Soma KK, Bindra RK, Gee J, Wingfield JC, Schlinger BA. 1999. Androgen-metabolizing enzymes show region-specific changes across the breeding season in the brain of a wild songbird. J Neurobiol 41: 176-188.

Soma KK, Hartman VN, Wingfield JC, Brenowitz EA. 1999. Seasonal changes in androgen receptor immunoreactivity in the song nucleus HVc of a wild bird. J Comp Neurol 409: 224-236.

Soma KK, Schlinger BA, Wingfield JC, Saldanha CJ. 2003. Brain aromatase, 5α-reductase and 5β-reductase change seasonally in wild male song sparrows: Relationship to sexual and aggressive behaviors. J Neurobiol 56: 209-221.

Soma KK, Sullivan K, Wingfield J. 1999. Combined aromatase inhibitor and antiandrogen treatment decreases territorial aggression in a wild songbird during the nonbreeding season. Gen Comp Endocrinol 115: 442-453.

Soma KK, Sullivan KA, Tramontin AD, Saldanha CJ, Schlinger BA, et al. 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 KK, Tramontin AD, Wingfield JC. 2000b. Oestrogen regulates male aggression in the non-breeding season. Proc Roy Soc London B 267: 1089-1096.

Soma KK, Wingfield JC. 1999. Endocrinology of aggression in the nonbreeding season. 22nd International Ornithological Congress. Adams N, Slotow R, editors. Durban, South Africa: University of Natal. pp. 1606-1620.

Soma KK, Wingfield JC. 2001. Dehydroepiandrosterone in songbird plasma: Seasonal regulation and relationship to territorial aggression. Gen Comp Endocrinol 123: 144-155.

Soma KK, Wissman AM, Brenowitz EA, Wingfield JC. 2002. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm Behav 41: 203-212.

Stavisky RC, Register TC, Watson SL, Weaver DS, Kaplan JR. 1999. Behavioral responses to ovariectomy and chronic anabolic steroid treatment in female cynomolgus macaques. Physiol Behav 66: 95-100.

Steklis HD, Brammer GL, Raleigh MJ, McGuire MT. 1985. Serum testosterone, male dominance, and aggression in captive groups of vervet monkeys (*Cercopithecus aethiops sabaeus*). Horm Behav 19: 154-163.

Steklis HD, Raleigh MJ, Kling AS, Tachiki K. 1986. Biochemical and hormonal correlates of dominance and social behavior in all-male groups of squirrel monkeys (*Saimiri sciureus*). Am J Primatol 11: 133-145.

Stribley JM, Carter CS. 1999. Developmental exposure to vasopressin increases aggression in adult prairie voles. Proc Natl Acad Sci USA 196: 12601-12604.

Strier KB, Ziegler TE, Wittwer DJ. 1999. Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoides*). Horm Behav 35: 125-134.

Suchowsky GK, Pegrassi L, Bonsignori A. 1969. The effect of steroids on aggressive behavior in isolated male mice. In: Garattini S, Sigg EB (eds). Aggressive Behaviour. Proc. Int. Symp. on Biology of Aggressive Behaviour. Amsterdam: Exerpta Medica Foundation, 164-171.

Suchowsky GK, Pegrassi L, Bonsignori. 1969. The effect of steroids on aggressive behaviour in isolated male mice.

Aggressive behaviour. Garattini S, Sigg EB, editors. New York: Wiley; pp. 164-171.

Tiefer L. 1970. Gonadal hormones and mating behavior in the adult golden hamster. Horm Behav 1: 189-202.

Tramontin AD, Brenowitz EA. 2000. Seasonal plasticity in the adult brain. Trends Neurosci 23: 251-258.

Uhrich J. 1938. The social hierarchy in albino mice. J Comp Pscyhol 25: 373.

van Goozen SHM, Matthys W, Cohen-Kettenis PT, Thijssen JHH, van Engeland H. 1998. Adrenal androgens and aggression in conduct disorder prepubertal boys and normal controls. Biol Psychiat 43: 156-158.

van Goozen SH, van den Ban E, Matthys W, Cohen-Kettenis PT, Thijssen JH, et al. 2000. Increased adrenal androgen functioning in children with oppositional defiant disorder: A comparison with psychiatric and normal controls. J Am Acad Child Adolesc Psychiat 39: 1446-1451.

Van Roo BL. 2004. Exogenous testosterone inhibits several forms of male parental behavior and stimulates song in a monogamous songbird: The blue-headed vireo (*Vireo solitarius*). Horm Behav 46: 678-683.

van Schaik CP, van Noordwijk MA, van Bragt T, Blankenstein MA. 1991. A pilot study of the social correlates of levels of urinary cortisol, prolactin, and testosterone in wild long-tailed macaques (*Macaca fascicularis*). Primates 32: 345-356.

von Engelhardt N, Kappeler PM, Heistermann M. 2000. Androgen levels and female social dominance in *Lemur catta*. Proc Roy Soc London B 267: 1533-1539.

Walker ML, Wilson ME, Gordon TP. 1983. Female rhesus monkey aggression during the menstrual cycle. Anim Behav 31: 1047-1054.

Walters MJ, Harding CF. 1988. The effects of an aromatization inhibitor on the reproductive behavior of male zebra finches. Horm Behav 22: 207-218.

Walters MJ, McEwen BS, Harding CF. 1988. Estrogen receptor levels in hypothalamic and vocal control nuclei in the male zebra finch. Brain Res 459: 37-43.

Weinbauer GF, Gockeler E, Nieschlag E. 1988. Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated non-human primate (*Macaca fascicularis*). J Clin Endocrinol Metabol 67: 284-290.

Whitsett JM. 1975. The development of aggressive and marking behavior in intact and castrated male hamsters. Horm Behav 6: 47-57.

Whitten PL. 2000. Evolutionary endocrinology of the cercopithecoids. Old World monkeys. Whitehead PF, Jolly CJ, editors. Cambridge: Cambridge University Press; pp. 269-297.

Whitten PL, Turner TR. 2004. Male residence and the patterning of serum testosterone in vervet monkeys (*Cercopithecus aethiops*). Behav Ecol Sociobiol 56: 565-578.

Wickings EJ, Dixson AF. 1992. Testicular function, secondary sexual development, and social-status in male mandrills (*Mandrillus sphinx*). Physiol Behav 52: 909-916.

Wiebe RH, Williams LE, Abee CR, Yeoman RR, Diamond EJ. 1988. Seasonal changes in serum dehydroepiandrosterone, androstenedione, and testosterone levels in the squirrel monkey (*Saimiri boliviensis boliviensis*). Am J Primatol 14: 285-291.

Wikelski M, Hau M, Wingfield JC. 1999. Social instability increases plasma testosterone in a year- round territorial neotropical bird. Proc Roy Soc London B 266: 551-556.

Wikelski M, Hau M, Wingfield JC. 2000. Seasonality of reproduction in a neotropical rain forest bird. Ecology 81: 2458-2472.

Wikelski M, Lynn S, Breuner C, Wingfield JC, Kenagy GJ. 1999. Energy metabolism, testosterone and corticosterone in white-crowned sparrows. J Comp Physiol A 185: 463-470.

Wiley CJ, Goldizen AW. 2003. Testosterone is correlated with courtship but not aggression in the tropical buff-banded rail, *Gallirallus philippensis*. Horm Behav 43: 554-560.

Wilson AP, Vessey SH. 1968. Behavior of free-ranging castrated rhesus monkeys. Folia Primatologica 9: 1-14.

Wingfield JC. 1984a. Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*. Vol. I. Temporal organization of the breeding cycle. Gen Comp Endocrinol 56: 406-416.

Wingfield JC. 1984b. Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*. Vol. II. Agonistic interactions as environmental information stimulating secretion of testosterone. Gen Comp Endocrinol 56: 417-424.

Wingfield JC. 1985. Short-term changes in plasma levels of hormones during establishment and defense of a breeding territory in male song sparrows, *Melospiza melodia*. Horm Behav 19: 174-187.

Wingfield JC. 1994. Control of territorial aggression in a changing environment. Psychoneuroendocrinology 19: 709-721.

Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF. 1990. The "challenge hypothesis": Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136: 829-846.

Wingfield JC, Hahn T. 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Anim Behav 47: 77-89.

Wingfield JC, Jacobs J, Hillgarth N. 1997. Ecological constraints and the evolution of hormone-behavior interrelationships. Ann NY Acad Sci 807: 22-41.

Wingfield JC. 1994. Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. Horm Behav 28: 1-15.

Wingfield JC, Ball GF, Dufty AM, Hegner RE, Ramenofsky M. 1987. Testosterone and aggression in birds. Am Sci 75: 602-608.

Wingfield JC, Farner DS. 1993. Endocrinology of reproduction in wild species. Avian biology. Farner DS, King J, Parkes K, editors. San Diego, CA: Academic; pp. 163-327.

Wingfield JC, Hegner RE, Dufty AM, Ball GF. 1990. The challenge hypothesis: Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136: 829-846.

Wingfield JC, Jacobs J, Hillgarth N. 1997. Ecological constraints and the evolution of hormone-behavior interrelationships. Integrative neurobiology of affiliation. Carter CS, Lederhendler II, Kirkpatrick B, editors. New York: New York Academy of Sciences; pp. 22-41.

Wingfield JC, Lynn SE, Soma KK. 2001. Avoiding the 'costs' of testosterone: Ecological bases of hormone-behavior interactions. Brain Behav Evol 57: 239-251.

Wingfield JC, Monk D. 1992. Control and context of year-round territorial aggression in the nonmigratory song sparrow, *Zonotrichia melodia morphna*. Ornis Scand 23: 298-303.

Wingfield JC, Sapolsky RM. 2003. Reproduction and resistance to stress: When and how. J Neuroendocrinol 15: 711-724.

Wingfield JC, Silverin B. 1986. Effects of corticosterone on territorial behavior of free-living male song sparrows, *Melospiza melodia*. Horm Behav 20: 405-417.

Wingfield JC, Soma KK. 2002. Spring and autumn territoriality in song sparrows: Same behavior, different mechanisms? Integr Comp Biol 42: 11-20.

Wingfield JC, Wada M. 1989. Changes in plasma levels of testosterone during male-male interactions in the song sparrow, *Melospiza melodia*: Time course and specificity of response. J Comp Physiol A 166: 189-194.

Whittingham LA, Schwabl H. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Anim Behav 63: 63-67.

Wommack JC, Salinas A, Melloni RH Jr, Delville Y. 2004. Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. J Neuroendocrinol 16: 767-775.

Wommack JC, Taravosh-Lahn K, David JT, Delville Y. 2003. Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters. Horm Behav 43: 229-236.

Wood RI. 1997. Thinking about networks in the control of male hamster sexual behavior. Horm Behav 32: 40-45.

Yen, HCY, Day DA, Sigg EB. 1962. Influence of endocrine factors on development of fighting behavior in rodents. Pharmacologist 4: 173.

Young J, Corpechot C, Perche F, Eychenne B, Haug M, Baulieu EE, Robel P. 1996. Neurosteroids in the mouse brain: Behavioral and pharmacological effects of a 3β hydroxysteroid dehydrogenase inhibitor. Steroids, 61: 144-149.

Zhang J-X, Zhang Z-B, Wang Z-W. 2001. Seasonal changes in and effects of familiarity on agonistic behaviors of rat-like hamsters (*Cricetulus triton*). Ecol Res 16: 309-317.

Zumpe D, Clancy AN, Bonsall RW, Michael RP. 1996. Behavioral responses to Depo-Provera, Fadrozole, and estradiol in castrated, testosterone-treated cynomolgus monkeys (*Macaca fascicularis*): The involvement of progestin receptors. Physiol Behav 60: 531-540.