

Bi-Directional Actions of Dehydroepiandrosterone and Aggression in Female Siberian Hamsters



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ABSTRACT

There is a well-established positive relationship between gonadal steroids and aggression. In some seasonally breeding species, however, aggression often persists or is increased during short "winter-like" days when the gonads are regressed and circulating levels of gonadal steroids are relatively low. Although the mechanisms underlying short-day increases in aggression are not fully known, the adrenal androgen dehydroepiandrosterone (DHEA) has been suggested as an alternative neuroendocrine mechanism regulating seasonal aggression. We used two complementary experimental approaches to examine the bi-directional actions of DHEA and aggression in female Siberian hamsters, a seasonal rodent that displays increased aggression concomitant with elevated circulating DHEA in short days. In Experiment 1, we examined the effects of aggressive interactions on DHEA concentrations before and after an aggressive encounter in long- and short-day hamsters. Serum DHEA was altered in a photoperiod-dependent manner, with decreased DHEA levels in response to aggression in short- but not long-day hamsters. Next, we experimentally induced adrenal DHEA release via injections of exogenous ACTH and assessed changes in aggressive behavior across photoperiods. We show a robust increase in aggression in short compared with long days during baseline aggression trials; however, aggression was not significantly increased further in response to ACTH in either photoperiod during post-ACTH aggression trials. These findings suggest that DHEA plays a role in the regulation of short-day aggression, while also highlighting the need for additional studies addressing the causal relationship between DHEA and aggression in this and others species. *J. Exp. Zool.* 325A:116–121, 2016. © 2015 Wiley Periodicals, Inc.

J. Exp. Zool.
325A:116–121,
2016

How to cite this article: Rendon NM, Demas GE. 2016. Bi-directional actions of dehydroepiandrosterone and aggression in female Siberian hamsters. *J. Exp. Zool.* 325A:116–121.

Studies of the neuroendocrine regulation of aggression have traditionally focused on the role of gonadal steroids, primarily testosterone (T), in mediating inter-male aggression; a majority of this work has been conducted using laboratory-bred strains of rats and mice [reviewed in: (Soma et al., 2015)]. Despite the traditional research focus on the role of T in the regulation of aggression in vertebrates, recent findings from a variety of study species and in experimental paradigms ranging from field to laboratory studies have proven this idea to be too simplistic [reviewed in: (Soma et al., 2015)]. It is becoming increasingly clear that steroid hormones in addition to T (e.g., dehydroepiandrosterone, DHEA) also play important roles in the regulation of aggressive behavior in both males and females either by acting independently from, or in conjunction with, T (Crews, '84; Moore et al., '85; Crews and

Grant sponsor: National Science Foundation; grant number: IOB-0543798; grant sponsor: National Science Foundation Doctoral Dissertation Improvement; grant number: IOS-1406063; grant sponsor: National Institutes of Health Training; grant number: T32HD049336.

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Received 28 October 2015; Revised 3 December 2015; Accepted 3 December 2015

DOI: 10.1002/jez.2001

Published online 24 December 2015 in Wiley Online Library (wileyonlinelibrary.com).

Moore, '86; Soma and Wingfield, 2001; Boonstra et al., 2008; Pradhan et al., 2008; Gutzler et al., 2009; Scotti et al., 2009).

Several studies have examined the neuroendocrine regulation of aggression in a seasonal context (Jasnow et al., 2000; Soma and Wingfield, 2001; Scotti et al., 2007; Boonstra et al., 2008; Pradhan et al., 2008; Gutzler et al., 2009). Many seasonally breeding animals maintain reproductive function during summer and curtail breeding during the winter. Ambient day length (photoperiod) serves as the primary cue used by many animals to coordinate seasonal responses (Walton et al., 2011). In Siberian hamsters (*Phodopus sungorus*), both males and females housed in short-“winter-like” days display increased aggressive behavior, despite reproductive quiescence and relatively low gonadal steroid levels (Jasnow et al., 2000; Scotti et al., 2007). The precise mechanisms underlying short-day aggression are not fully known, but appear largely independent of gonadal steroids [reviewed in: (Soma et al., 2015)]. For example, gonadectomy and administration of exogenous gonadal steroids to short-day male or female hamsters does not affect aggression (Jasnow et al., 2000; Scotti et al., 2007). These findings suggest alternative neuroendocrine mechanisms, including non-gonadal (e.g., adrenal gland, brain) steroids that are known regulators of aggression (Soma and Wingfield, 2001; Boonstra et al., 2008; Pradhan et al., 2008; Gutzler et al., 2009; Scotti et al., 2009; Soma et al., 2015).

There is compelling evidence in both male and female hamsters that short-day aggression is regulated by the adrenal glands. Increased aggression in short-day-like animals is blocked by bilateral adrenalectomy (which eliminates both adrenal steroids and catecholamines), but not demedullation (which eliminates catecholamines, leaving adrenal steroids intact) (Demas et al., 2004). Although these findings suggest a role for adrenal steroids, short-day aggression is independent of both cortisol (CORT) levels and glucocorticoid receptor expression in hamsters (Scotti et al., 2015). In contrast, the adrenal androgen DHEA, a steroid that serves as a prohormone and can be converted to biologically active hormones (e.g., T and 17 β -estradiol (E₂)), appears to be a critical regulator of aggression in this and other species (Soma and Wingfield, 2001; Pradhan et al., 2008; Scotti et al., 2009; Rendon et al., 2015). Short-day females display a more robust adrenal DHEA response to exogenous adrenocorticotrophic hormone (ACTH), a potent secretagogue of both DHEA and CORT, compared with long-day hamsters (Rendon et al., 2015). Further, ACTH-induced increases in DHEA, but not CORT levels, are correlated with aggression but only in short days, suggesting DHEA-specific responsiveness and association in short days (Rendon et al., 2015).

The goal of the present study was to examine the potential bi-directional actions of adrenal DHEA release and aggression within a seasonal context in female hamsters. We examined this using two complementary experimental approaches. In experiment 1, we exposed long and short day hamsters to an aggressive encounter and examined changes in serum DHEA concentrations. In experiment 2, we recorded both baseline and post-ACTH levels

of aggression. We experimentally induced DHEA release in females from both photoperiods via injections of exogenous ACTH (i.e., ACTH challenges) and assessed changes in aggressive behavior. Although both sexes show increased aggression in short days, we chose to focus on females because considerably less is known regarding the neuroendocrine regulation of female aggression in this and other rodent species.

MATERIALS AND METHODS

Animal Housing

Adult (>60 days of age) female Siberian hamsters (*Phodopus sungorus*) were derived from a breeding colony maintained at Indiana University. Hamsters were bred and housed under long days (light:dark, L:D 16:8 hr), and group-housed at weaning (postnatal day 18). Ambient temperature was maintained at 20 \pm 2°C, relative humidity was maintained at 55 \pm 5%, and hamsters were given *ad libitum* access to filtered water and laboratory rodent chow (Lab Diet 5001, PMI Nutrition). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University.

Photoperiodic Manipulations

Hamsters were individually housed for a 1 week acclimation period under long days. Following acclimation, a subset of hamsters were selected and transferred to short days (L:D 8:16 hr); the remaining hamsters were kept in long days. Both long- and short-day animals received this photoperiodic treatment for ten weeks in order to induce reproductive and non-reproductive phenotypes. Females were classified as reproductive or non-reproductive based on well-established *a priori* criteria for this species (Rendon et al., 2015; Scotti et al., 2007). Long-day reproductive females displayed estrous cyclicity, had functioning ovaries, displayed no significant change in body mass and maintained brown pelage. In contrast, short-day non-reproductive females were characterized as such if they were anestrus or acyclic, had regressed ovaries, lost >10% of their body mass, and had white pelage (Rendon et al., 2015; Scotti et al., 2007). A subset of short-day females (i.e., non-responders) failed to respond reproductively to changes in photoperiod, a phenomenon that has been documented in this species (Lynch et al., '89; Scotti et al., 2007; Rendon et al., 2015). These females ($n = 3$) were excluded from the experiments due to insufficient numbers for statistical analyses.

Experiment 1: Effects of Aggression on DHEA Release

After 10 weeks of photoperiodic treatment, pre-aggression baseline blood samples were collected, and all hamsters underwent territorial aggression trials. Aggression was measured in all animals (long days, $n = 9$; short days, $n = 17$), using a 5 min resident-intruder paradigm described previously (Rendon et al.,

2015). Staged female dyads were created, composed of an experimental animal (i.e., resident) and a stimulus animal (i.e., intruder) of approximately the same age, with comparable ($\pm 5\%$) body mass and from different parents. The intruder was introduced to the resident's home cage, which had not been changed for 7 days prior to the interaction to allow the resident hamster to establish its territory, therefore, facilitating high aggression from the resident hamster. Blood samples were collected 30 min following inception of behavioral interaction (i.e., 25 min post-aggression trial). Aggression (i.e., latency to first attack (sec), number and duration of attacks, and number and duration of chases) was quantified for experimental animals. No behavioral measures were quantified in stimulus animals. Serum DHEA was quantified using a commercially available enzyme immunoassay (EIA; ALPCO Diagnostics, 20-DHEHU-E01) as outlined previously for Siberian hamsters (Rendon et al., 2015). Intra-assay variability was 5.51%.

Experiment 2: Effects of DHEA Release on Aggression

An additional group of female hamsters ($n = 30$) underwent photoperiodic manipulations as above. After 10 weeks of photoperiodic treatment, baseline blood samples were collected, and all hamsters underwent two territorial aggression trials (i.e., a baseline aggression trial, and a post-ACTH treatment aggression trial). Aggression was measured in all resident animals (long days, $n = 14$; short days, $n = 16$), as described above. Three days following the first behavioral testing (to minimize the effects of the initial behavioral trial on the subsequent trial), both long- and short-day animals were randomly assigned to either an ACTH injection (long days, $n = 8$; short days, $n = 8$) or a control (saline) injection (long days, $n = 6$; short days, $n = 8$) group to assess the effect of DHEA elevation on aggression. Animals received an i.m. injection of either 4IU/kg of synthetic ACTH (Cortrosyn, Henry Schein Animal Health, Melville, NY, USA) or a saline control. The ACTH dose and time point was chosen based on a previously used protocol in Siberian hamsters (Rendon et al., 2015), and red squirrels (Boonstra et al., 2008). This protocol has been shown to significantly elevate both DHEA and CORT in hamsters (Rendon et al., 2015). Following injection of ACTH or saline, the animals were returned to their home cages and remained undisturbed. After 30 min, a second aggression trial was performed. Baseline levels of serum DHEA were quantified as above; intra-assay variability was 1.65%.

STATISTICAL ANALYSES

All statistical analyses were performed in JMP v. 12.0.0 (SAS Institute, Inc., Cary, NC, USA) and statistical significance was reported if $P < 0.05$. Data were transformed to attain normality and homogeneity of variances. Two-tailed t -tests were used to compare physiological and behavioral changes across photoperiodic treatments. Mixed model analyses of variance (ANOVAs) were calculated with photoperiod as a between-groups variable

and time (pre- vs. post-DHEA or aggression) as a within-subjects variable. If an ANOVA reported a significant effect or interaction of effects pair-wise comparisons were conducted using two-tailed t -tests. Repeatability of aggression of each individual was calculated as described previously (Lessells and Boag, '87).

RESULTS

Experiment 1: Effects of Aggression on DHEA Release

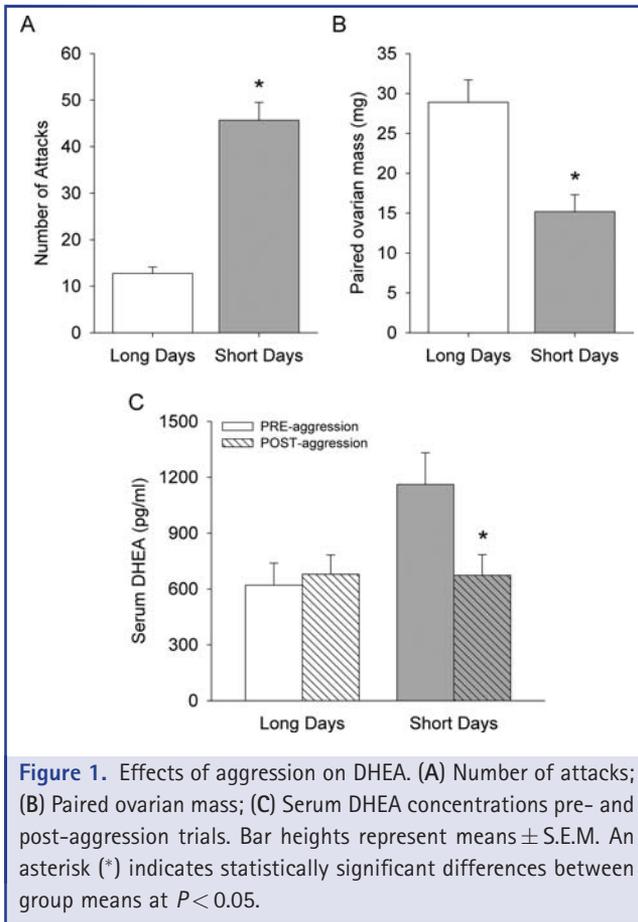
Short-day female hamsters displayed more ($t_{25} = 8.09$, $P < 0.001$; Fig. 1A) and longer attacks ($t_{25} = 4.16$, $P = 0.003$), and more ($t_{25} = 5.05$, $P < 0.001$) and longer chases ($t_{25} = 6.20$, $P < 0.001$), compared with long-day animals. There was no photoperiodic effect on latency to first attack ($t_{25} = -1.92$, $P = 0.08$). Short-day females also displayed decreased paired ovarian masses ($t_{25} = -3.90$, $P = 0.004$; Fig. 1B), decreased body masses (-20% , $t_{25} = -6.13$, $P < 0.001$), and higher circulating DHEA ($t_{25} = 3.35$, $P = 0.002$), compared with long-day females. Short- but not long-day females exhibited decreased DHEA in response to an aggression challenge ($F_{1,25} = 6.24$, $P = 0.02$; Fig. 1C).

Experiment 2: Effects of DHEA Release on Aggression

Short-day female hamsters displayed higher circulating DHEA ($t_{29} = 1.92$, $P = 0.03$; Fig. 2A), decreased paired ovarian masses ($t_{29} = -3.14$, $P = 0.005$; Fig. 2B), and decreased body masses (-24% , $t_{29} = 10.37$, $P < 0.001$), compared with long-day females. Short-day females displayed more ($F_{1,29} = 33.44$, $P < 0.001$; Fig. 2C) and longer attacks ($F_{1,29} = 11.03$, $P = 0.002$), and more ($F_{1,29} = 23.66$, $P < 0.001$) and longer chases ($F_{1,29} = 13.07$, $P = 0.001$), compared with long-day animals. There was no photoperiodic effect on latency to first attack ($F_{1,29} = 9.85$, $P = 0.17$). Aggression was not further increased in response to an ACTH challenge in either photoperiod; there was no treatment effect on number of attacks ($F_{1,29} = 3.96$, $P = 0.64$; Fig. 2C), duration of attacks ($F_{1,29} = 3.92$, $P = 0.77$), number of chases ($F_{1,29} = 2.61$, $P = 0.65$), duration of chases ($F_{1,29} = 3.44$, $P = 0.82$), and latency to first attack ($F_{1,29} = 3.67$, $P = 0.45$). Aggression across both behavioral trials was highly repeatable ($F = 2.28$; $r = 0.95$; $P = 0.02$), indicating that aggression is reliable within individuals and consistently different among individuals.

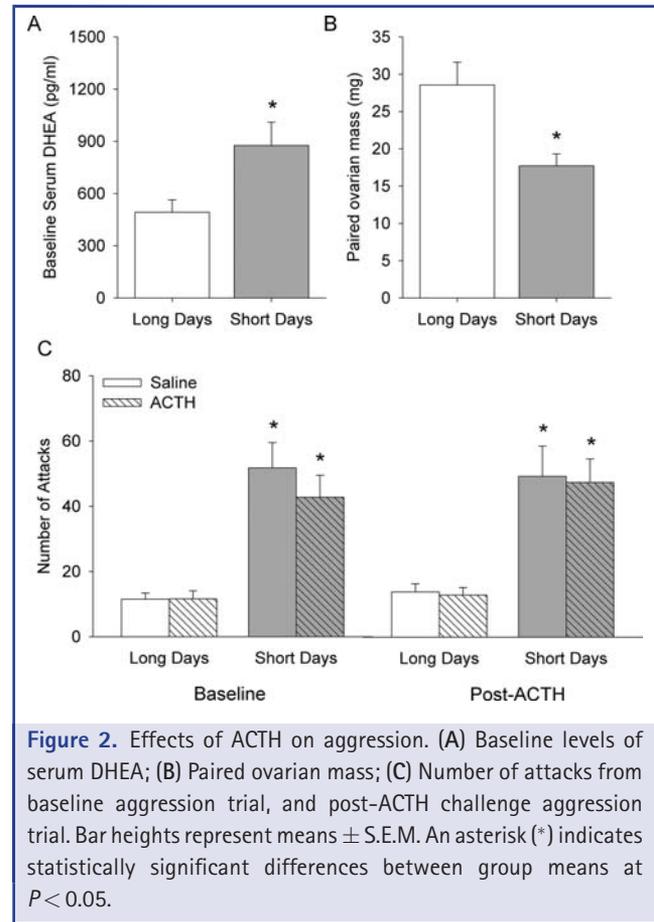
DISCUSSION

In Experiment 1 we show that aggressive challenges affect DHEA responses differently across photoperiodic conditions. Specifically, short- but not long-day animals showed a decrease in DHEA post-aggression, similar to what has been shown in male hamsters (Scotti et al., 2009). These data are consistent with the idea that DHEA serves as a prohormone and that exposure to short days facilitates conversion of DHEA to other bioactive steroids such as T and E_2 . Whereas neither T nor E_2 were measured in the current studies due to insufficient blood samples, we have previously



shown that short- but not long-day females show increased serum E_2 levels following an aggression trial compared with baseline E_2 levels, suggesting behaviorally-induced conversion of DHEA to E_2 (N.M. Rendon & G.E. Demas, unpublished data). The enzyme 3β -hydroxysteroid dehydrogenase/isomerase (3β -HSD) catalyzes the conversion of DHEA to androstenedione, which can then be converted to T by 17β -hydroxysteroid dehydrogenase (17β -HSD), and subsequently to E_2 via aromatase. Increased 3β -HSD activity would be consistent with the pattern of a rapid decrease in DHEA post-aggression, and suggest a photoperiod-dependent role of 3β -HSD in converting DHEA from a prohormone to a biologically active steroid, which in turn could regulate aggression. In support of this idea, brain 3β -HSD activity is higher in winter than in spring in song sparrows (Pradhan et al., 2008). Future studies will directly test the short-day specific enzymatic conversion of DHEA to other biologically active steroids, and its role in regulating seasonal aggression in hamsters.

In Experiment 2 we show a robust increase in aggression in short days when compared with long days, confirming our previous findings (Jasnow et al., 2000; Scotti et al., 2007; Rendon et al., 2015); however, a mixed model ANOVA did not reveal a



significant increase in aggression in response to ACTH in either photoperiod. To probe the seeming lack of effect of ACTH on aggression, we calculated effect sizes using Cohen's d for the change in number of attacks from baseline aggression to post-ACTH aggression across photoperiods. Interestingly, we found that the long-day control ($d = 0.13$), short-day control ($d = 0.14$), and long-day ACTH groups ($d = 0.18$) all had low effect sizes that were indistinguishable from one another. In contrast, there was a doubling of the effect size for the short-day ACTH group ($d = 0.28$), reflecting a medium effect size. These findings support the hypothesis that exogenous ACTH may have a modest effect of increasing aggression in a short-day specific manner. One possible explanation for this modest effect is that female hamsters may have been at their capacity to respond aggressively, and thus did not exhibit a significant increase in aggression in response to an ACTH challenge (i.e., a "ceiling effect"). The resident-intruder paradigm typically employed in this and other studies maximizes aggressive responses and thus might not allow for greater increases in aggression to be detected. Studies using different behavioral paradigms (e.g., neutral arena) to decrease the overall levels of aggression displayed by the residents could

be useful in further investigating this modest effect of ACTH increasing aggression.

There are alternative hypotheses that future studies could address. One alternative hypothesis is that that marked ACTH-induced increases in CORT concentrations attenuated the actions of elevated DHEA concentrations on aggression (Rendon et al., 2015). Prior studies have shown that elevated glucocorticoid levels can inhibit aggression in this and other species (Nock and Leshner, '76; Leshner et al., '80; Wingfield and Silverin, '86; Scotti et al., 2015). Studies that block hormone-specific enzymatic cascades allowing for differential release of DHEA and CORT concentrations in response to an ACTH challenge could distinguish these likely effects. Another alternative hypothesis is that a single injection of ACTH in the absence of an actual social interaction may be insufficient to induce aggression due to the lack of requisite social and endocrine priming. Social interactions have the ability to prime endocrine systems such that subsequent responses are more pronounced (Gleason et al., 2009; Wingfield et al., '90). For example, in territorial California mice (*Peromyscus californicus*), winning in the home cage (Fuxjager et al., 2009), and repeated post-victory surges in androgens, increase the likelihood of winning (Fuxjager et al., 2011). Bi-directional feedback of behavior and physiology are hypothesized to prepare animals for future social encounters [reviewed in: (Rosvall and Peterson, 2014)]. Thus, it is possible that ACTH challenges, in the absence of actual social interactions, are not sufficient to elicit further increases in aggression.

Regardless of the precise mechanisms, the present study provides support for the idea that DHEA and aggression co-vary in a photoperiod-dependent manner. These results, coupled with previous findings in birds, rodents, and humans linking DHEA to aggression (Van Goozen et al., '98; Soma and Wingfield, 2001; Boonstra et al., 2008; Pradhan et al., 2008; Gutzler et al., 2009; Scotti et al., 2009; Rendon et al., 2015), suggest that DHEA remains a viable endocrine candidate for the regulation of aggression. It is also clear, however, that future studies are needed to determine the precise causal relationship between DHEA and aggression in Siberian hamsters as well as other species.

ACKNOWLEDGMENTS

The authors thank L.K. Achury, A.C. Amez, K.J. O'Malley, and E.R. Weigel for assistance in behavioral filming, necropsies, and general animal procedures. Thanks to E.R. Weigel for scoring behavioral videos for inter-rater reliability, and L.R. Wright for analyzing estrous slides. This work was supported by National Science Foundation Grant IOB-0543798 to G.E.D., National Science Foundation Doctoral Dissertation Improvement Grant IOS-1406063 to N.M.R and G.E.D., National Institutes of Health Training Grant T32HD049336—"Common Themes in Reproductive Diversity" to N.M.R., The Society for Integrative and Comparative Biology Grant-in-Aid of Research award to N.M.R., and Indiana University.

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