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The role of androgens in the mediation of seasonal territorial aggression in male Siberian hamsters (*Phodopus sungorus*)

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ABSTRACT

Testosterone (T) mediates aggression in a wide range of species. In some species, however, aggressive behavior persists or increases during the non-breeding season when T levels are relatively low. Animals that do not display a positive correlation between aggression and gonadal steroids suggest the need for further investigation of alternative neuroendocrine mechanisms mediating seasonal aggression. Siberian hamsters (*Phodopus sungorus*) are an ideal study system because they display increased territorial aggression during the non-breeding season which may be independent of circulating T levels. The goals of the present study were to: 1) explore the role of T in the aggression of reproductive males, and 2) test the hypothesis that the adrenal steroid dehydroepiandrosterone (DHEA) acts as an endocrine regulator of seasonal aggression. In Experiment 1, individuals were housed in long day (breeding) photoperiod and received castrations, exogenous T capsules or both manipulations. In Experiment 2, animals were housed in either long or short days (non-breeding) photoperiod and received DHEA or empty capsules. In both experiments, serum hormone levels and aggressive behavior were assessed. In Experiment 1, castration did not reduce aggression whereas exogenous T actually inhibited aggressive behavior. In Experiment 2, short-day individuals were more aggressive than long-day animals but DHEA treatment did not affect aggressive behavior, regardless of photoperiod. The present study supports the hypothesis that circulating gonadal steroids are not necessary to activate aggressive behavior in adult male hamsters. Further, seasonal changes in territorial aggression appear independent of circulating levels of DHEA in Siberian hamsters.

1. Introduction

Aggression is a ubiquitous behavioral adaptation that has been extensively studied and well-characterized in a multitude of species and across a variety of contexts [1]. Aggression is typically exhibited when the interests of two or more individuals conflict, often over limited resources (e.g. mates, food, territories). Thus, aggression may confer a survival advantage to animals, when resources are low and the competition for those resources is high. Elevated territorial aggression is most often observed during the breeding season when individuals (typically males) compete for access to resources that will increase their opportunities for reproductive success [2]. The precise mechanisms underlying the regulation of aggressive behavior, however, are still not completely understood.

A large number of studies of male aggressive behavior have demonstrated a significant positive correlation between circulating levels of gonadal steroids, specifically testosterone (T), and territorial aggression. For example, extensive studies of rodent species such as rats (*Rattus norvegicus*), house mice (*Mus musculus*), gerbils (*Meriones unguiculatus*) and Syrian hamsters (*Mesocricetus auratus*) have demonstrated that male–male aggression can be reduced by castration and restored by exogenous T replacement [3–7]. Further, often times individuals with higher endogenous levels of T can be more aggressive and may be dominant over conspecifics with lower circulating T levels regardless of aggression levels [8,9]. This relationship between T and aggression is not exclusive to mammals and is observed in a variety of vertebrate taxa (e.g., fish, birds, reptiles) [10–12].

In some seasonal species, however, the positive relationship between T and aggression is not maintained throughout the year. For example, dusky footed wood rats (*Neotoma fuscipes*), song sparrows (*Melospiza melodia*), and European stonechats (*Saxicola rubicola*) maintain high levels of aggressive behavior outside of the breeding season, when circulating T levels are relatively low and often castration and/or androgen blockade has no effect on aggression during this time in free living individuals [13–15].

A particularly striking example of species in which non-breeding aggression is elevated relative to breeding aggression is that of Syrian and Siberian (*Phodopus sungorus*) hamsters. In hamsters breeding occurs during the summer months [16]. During the winter months these animals cease breeding and undergo gonadal regression [16]. The seasonal changes that have been observed in the field can reliably be mimicked in the laboratory by exposing animals to seasonally-

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appropriate photoperiods [16–19]. When exposed to long "summer-like" breeding photoperiods reproductive condition is maintained; however, when housed under short "winter-like" non-breeding photoperiods, gonadal regression occurs [17,18]. Interestingly, elevated levels of offensive aggression and/or increases in the ratio of offensive behavior to defensive behavior are observed during the winter non-breeding period when the gonads have regressed and gonadal steroid levels are relatively low in both of these species [17,18,20–22]. Previous studies of photoperiodic effects on the aggressive behavior of Siberian hamsters have reported that aggression is independent of or, perhaps, inversely related to circulating testosterone concentrations during short days [18].

The lack of a strong positive link between circulating T and aggression in Siberian hamsters suggests the need for further investigation of possible alternative hormonal mechanisms mediating aggressive behavior [23,24]. Previous research suggests that adrenocortical steroids may play a crucial role in aggressive behavior [25–27]. In support of this idea, short-day-like increases in aggressive behavior are eliminated by adrenalectomy [28,29]. Disruption of the adrenomedullary catecholamines (via adrenal demedulation), in contrast, has no effect on aggression [28]. These results support the hypothesis that adrenocortical steroids play a role in mediating territorial aggression; however, it is not currently known what class of cortical steroids, glucocorticoids or adrenal androgens (i.e. dehydroepiandrosterone (DHEA)), are mediating aggressive behavior.

Although there are substantial data to suggest that glucocorticoids affect aggressive behavior [30–37], considerably less work has been done investigating the role of DHEA in this capacity. Several recent studies suggest a role for DHEA in the mediation of non-breeding aggression. For example, male song sparrows (*M. melodia morphana*) captured during the non-breeding season, after initial behavioral observation, and implanted with physiologically relevant doses of DHEA [38] increased the number of songs and significantly reduced song latency in response to a simulated territorial intrusion (STI) [38]. Both male and female spotted antbirds (*Hylophylax n. naevioides*) are also aggressive during the non-breeding season (i.e., when gonads are regressed). Plasma DHEA levels are detectable and higher than that of T and estradiol (E_2) in both sexes, and plasma DHEA levels in males are positively correlated with aggressive vocalizations [39].

Evidence for a role of DHEA in mediating mammalian aggression is considerably more limited than for birds. Preliminary studies in male Siberian hamsters have indicated that DHEA levels are elevated in short-day housed individuals [40]. Studies in mice and rats, however, have also found that DHEA may suppress aggressive behavior [41–43]. Although these data suggest a possible role for DHEA in regulating aggression, the specific role of DHEA in aggressive behavior is equivocal and requires further study.

The goal of the current study was to investigate the potential role of gonadal and adrenal androgens, specifically T and DHEA, in the mediation of seasonal aggression Siberian hamsters. Specifically, we hypothesized that: 1) gonadal steroids are not required to activate aggressive behavior in long-day housed males and that 2) DHEA mediates the increases the territorial aggression observed in short-day Siberian hamsters. We predict that short-day individuals will be more aggressive than long-day individuals in both studies, neither castration nor T treatment will affect aggressive behavior, and that DHEA treatment will increase aggression in both long-day and short-day animals.

2. Methods

2.1. Animals and housing conditions

Adult (>60 days of age) male Siberian hamsters (*P. sungorus*) were obtained from our breeding colony. One week before the start of the experiments, hamsters were housed individually in polypropylene cages (27.8×17.5×13.0 cm) in colony rooms with a 24 h light: dark 16:8 cycle (lights off 1800 h EST). Temperature was kept constant at 20 ±2 °C and relative humidity was maintained at 50±5%. Food (Purina Rat Chow) and tap water were available *ad libitum* throughout the experiment. Additional animals were used as non-aggressive intruders during behavioral testing and were group-housed (three to four animals per cage) in long days (light: dark 16:8) to keep aggression to a minimum [44–46]. These animals were approximately two months younger than experimental animals. These individuals are typically smaller than the long-day residents. This does not hold in the cases of short-day residents vs. long-day intruders. Siberian hamsters typically lose 10% of their body weight when moved into short-day photoperiod [47]. In the case of such interactions the intruder is always younger than the resident but may in fact be larger. These animals were chosen to facilitate aggression from the resident [18]. Non-aggressive intruders were used no more than twice per test day. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and a protocol previously approved by the Bloomington Institutional Animal Care and Use Committee (BIACUC).

2.2. Experimental design

2.2.1. Experiment 1: The role of gonadal steroids in the aggression of long-day males

Adult male Siberian hamsters (n=45) were weighed and individually housed under long-day photoperiod conditions (light: dark 16:8 h) at the beginning of the experiment. Animals were then randomly assigned to one of 4 treatment groups castration with T replacement (Cast+T) (n=12), castration with no replacement (Cast) (n=11), sham castration plus T treatment (Sham+T) (n=11), and sham castration with no T replacement (Sham) (n=11). 4 weeks after the surgeries behavioral trials were run. All animals were tested using a resident–intruder model of aggression by introducing a non-aggressive intruder into the home cage of an experimental animal for 5 min to assess territorial aggression [18]. Behavior was tested 2 h prior to lights off relative to the respective photoperiods (i.e., 1400 for short days and 1800 for long days) to control for circadian rhythmicity of both behavior and hormone levels. Although hamsters typically display increased aggressive behavior during the dark phase, animals were tested during the light phase to reduce the likelihood of physical injury to the animals. Further, we have previously demonstrated that photoperiodic changes in aggression occur regardless of whether animals are tested during the dark or light phase (M.S. Scotti, G.E. Demas, unpublished). All trials were performed under low illumination/red light conditions, which allowed for the display of natural behaviors of the hamsters while providing sufficient light to allow for video recording and observations. Intruders were identified by small patches of shaved fur on their dorsal surfaces. To facilitate the establishment of territoriality, the bedding material of experimental animals remained unchanged for at least one week prior to behavioral testing [18]. Twenty four hours prior to behavioral testing, resident hamsters were lightly anesthetized with diethyl ether (VWR, Indianapolis, IN) and blood samples were drawn from the retro-orbital sinus. Serum was extracted following centrifugation. Behavioral interactions were videotaped and scored using ODlog™ software (Macropod Software) by an observer naïve to experimental conditions. Aggressive, social, and non-social behaviors were characterized.

2.3. Castrations

Castrations in Experiment 1 were performed as described previously [18]. Briefly, hamsters were anesthetized with 0.06 cm^3 of a 1:10 ketamine cocktail comprised of ketamine (100 mg/ml; Henry Schein, Indianapolis, IN), xylazine (20 mg/ml; Henry Schein, Indianapolis, IN),

and 0.9% saline. Castrations were performed through bilateral abdominal incisions; both testes were removed, the abdominal wall sutured, and the incisions in the skin were closed with 9-mm wound clips. The wounds were treated with nitrofurazone antibacterial ointment (0.2% nitrofurazone; Squire Laboratories Inc., Revere, MA). Hamsters undergoing sham castrations received a similar procedure, except the testes were simply visualized prior to closing. Individuals in Experiment 1 were implanted with silastic capsules post surgery but while still anesthetized.

2.4. Hormonal manipulation

Cast+T and Sham+T individuals were implanted with 10-mm long Silastic capsule implants (1.47-mm inner diameter, 1.95-mm outer diameter, silicone medical grade tubing; Dow Corning Co., Midland, MI) filled with T (Sigma T-1500; Sigma-Aldrich, St. Louis, MO) [18]. Cast and Sham individuals were implanted with empty Silastic capsule implants.

To implant the capsules a 70% alcohol solution and betadine were applied to the interscapular surface and a 5 mm incision was made perpendicular to the midline [18,22]. Capsules were implanted subcutaneously and the incision was closed with a 9-mm wound clip. In addition a surface antibiotic (0.2% nitrofurazone; Squire Laboratories Inc., Revere, MA) was applied to the wound after the implantation. Animals remained in their respective photoperiodic conditions for four weeks before behavioral testing, and their cages were not changed for one week prior to behavioral testing.

2.4.1. Experiment 2: The role of DHEA in the mediation of seasonal aggression

Adult male Siberian hamsters ($n=40$) were weighed and individually housed under either a light: dark 16:8 h ($n=17$) or a light: dark 8:16 h cycle ($n=22$) at the beginning of the experiment. At the end of 8 weeks, the animals were re-weighed. In many seasonally breeding rodents, including Siberian hamsters, there is a small subset of individuals that are non-responsive to short-day photoperiods. These photoperiodic "non-responders" do not undergo gonadal regression and generally respond physiologically and behaviorally like long-day animals. A typical Siberian hamster that is responsive to short-day photoperiod loses at least 10% of its body weight [47]. Any animals that did not lose weight or gained weight while housed in short-day photoperiod were placed in the non-responder group and removed from the study ($n=7$). Individuals that were long-day housed ($n=18$) and short-day responders ($n=17$), were then randomly assigned to receive either control treatment or DHEA treatment. Upon necropsy any short-day animal with a paired testes weight of less than 0.12 g was assumed to be a responder [48]. Behavioral trials were run similarly to Experiment 1.

2.5. Hormonal manipulations

In Experiment 2 approximately half of the long-day housed animals ($n=9$) and short-day housed animals ($n=7$) were selected randomly and were implanted with 10-mm long Silastic capsule implants (1.47-mm inner diameter, 1.95-mm outer diameter, silicone medical grade tubing) filled with DHEA (Sigma D4000). Control animals (long-day $n=9$ and short-day $n=8$) were implanted with empty Silastic capsule implants. One short-day+DHEA animal lost its capsule and was removed from the study. Animals were lightly anesthetized with diethyl ether prior to the procedure. An analgesic, metacam (1.5 mg/ml meloxicam; Vetmedica Inc., St. Joseph, MO), was given orally after the surgery. All other implant surgical procedures were identical to those in Experiment 1. Animals were returned to photoperiod for two weeks before behavioral testing, and their cages were not changed for one week prior to behavioral testing.

2.6. Blood collection

For both Experiments 1 and 2, 24 h prior to the behavioral testing animals were lightly anesthetized and blood samples were drawn into capillary tubes via retro-orbital bleeding. Handling time was kept consistent and at a minimum; typically less than 3 min elapsed between removal from the cage to return to the home cage. Blood samples were allowed to clot for 1 h, the clots were removed and the samples centrifuged (at 8 °C) for 30 min at 2500 rpm. Serum aliquots were aspirated and then stored in sealable polypropylene microcentrifuge tubes at −20 °C until assayed.

2.7. Behavioral measures

In both Experiments 1 and 2 a resident–intruder model of aggression was used, consisting of the placement of a non-aggressive long-day male hamster into the home cage of a singly housed resident/experimental animal for 5 min [18]. Intruder animals were adult males younger and smaller (but see above) than residents/experimental animals. The following behaviors were scored over the 5 min testing period: aggressive behaviors (i.e., chasing and attacking), investigative behavior (i.e., anogenital investigation) and non-social behaviors (i.e., grooming and attempting to climb out of the cage (escape)). We defined attack as physical contact between the resident and intruder that was initiated by the resident and resulted in biting and/or pinning. We considered an interaction a chase when the resident pursued a fleeing intruder eventually resulting in an attack. Anogenital investigation (AGI) was scored as the resident sniffing the anogenital region of the intruder. In addition to the number of attacks, the latency to initial attack was also quantified [18].

2.8. Necropsies

Upon completion of behavioral trials in both studies, all animals were euthanized via 0.4 cm^3 of a 1:10 ketamine cocktail comprised of ketamine (100 mg/ml) xylazine (20 mg/ml), and 0.9% saline. Necropsies were performed on all animals and implants were removed to ensure hormone remained in those that had been filled. Paired testes were collected from animals in Experiment 2, cleaned of fat and connective tissue and weighed. Animals that after 10 weeks in short days, had paired testes weighing >0.12 g were classified as short-day non-responders; animals with paired testes weighing <0.12 were classified as short-day responders.

2.9. Hormone assays

For both studies, total serum T was measured in duplicate using an EIA kit produced by Assay Designs (Ann Arbor, MI). The sensitivity for this assay is 5.67 pg/ml. Cross-reactivities with androstenedione (AE), DHEA and estradiol (E_2) were 7.20%, 0.72%, 0.40% respectively. Serum samples were diluted 1: 20 with assay buffer. The intra assay variation for this was 3.63%. Total serum DHEA was measured in Experiment 2 in duplicate using an EIA kit produced by Diagnostic Systems Laboratories (Ann Arbor, MI). The sensitivity for this assay is 0.1 ng/ml. Cross-reactivity with AE is 0.27%. Cross-reactivities with all other hormones, including DHEA-S, are non-detectable. Serum samples were diluted 1:2 with the 0 standard. The intra assay variation for this was 13.25%. Samples that did not fall along the standard curve were not used in statistical analyses.

2.10. Statistical analyses

In Experiment 1 a 2 (surgery type)×2 (hormone treatment) ANOVA was used when the results did not violate normality and equal variance. When these assumptions were violated, natural log transformations were performed. Data for serum T, latency, escape,

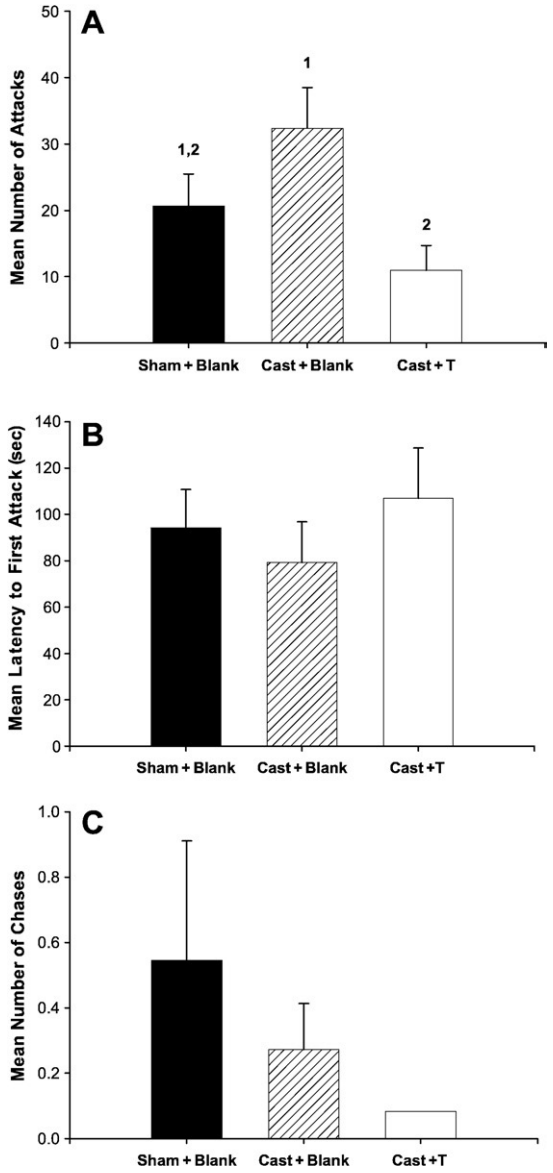

Fig. 1. Mean (±SEM) number of attacks (A), latency to first attack (B) and number of chases (C) for castrated (Cast) or sham-castrated hamsters and implanted with empty (Blank) capsules or testosterone filled (T) capsules. Bars sharing at least one number in common are statistically equivalent. Bars with different numbers are statistically different at $p<0.05$.

grooming, were successfully transformed. When transforming the data did not resolve these problems then non-parametric tests (Kruskal–Wallis test) was used (attack and chase). A one-way Welch's ANOVA was used to analyze T concentration for Experiment 1. A Tukey's post hoc test was performed to further probe main effects. To ameliorate the possible effects of supra-physiological doses of T in the Sham+T group on the statistical analyses, the experimental data was re-analyzed excluding this group. A one-way ANOVA was used to analyze data, from Experiment 1 after the removal of the Sham+T group from the analyses. Data for serum T levels, escape, and grooming were log transformed. Tukey's post hoc tests were performed to further probe main effects.

In Experiment 2 a 2 (photoperiod)×2 (hormone treatment) ANOVA was used. Attack, latency, serum T, serum DHEA and grooming, data were transformed via natural log when they did not conform to assumptions of normality and/or equal variance. If this did not rectify the problem, Mann–Whitney tests were employed (chase, adrenal weights, gonad weights). Differences among means were considered significant if $p<0.05$. Analyses were performed using the SPSS (Chicago, IL) statistical package.

3. Results

3.1. Experiment 1

As predicted, castration had no effect on aggressive behavior ($Z=-0.57$, $p>0.05$). The type of implant, however, had a significant effect on aggressive behavior; individuals that received T capsules were significantly less aggressive than those that received empty capsules ($Z=2.78$, $p=0.005$). There was no effect of surgery ($F_{1,41}=0.987$, $p=0.326$) or hormone treatment ($F_{1,41}=2.375$, $p=0.131$) on latency to first attack, however, as expected, latency to first attack and attack number were negatively correlated ($R=-0.472$, $p=0.001$). The number of attacks was also negatively correlated with serum T concentrations ($R=-0.376$, $p=0.020$). Treatment significantly affected T concentration ($F_{3,18.516}=22.105$, $p<0.001$). Post hoc analysis show that individuals that were castrated but did not receive T replacement (Cast) had significantly lower T levels than Sham, Sham+T and Cast+T animals ($p=0.001$, $p<0.001$, $p<0.001$ respectively). All other groups had T levels that did not differ significantly ($p>0.05$). There was no effect of either treatment on any other behavioral measure (chase, AGI, grooming) ($p>0.05$) except escape/climbing behavior. Individuals that received exogenous T tried to escape the cage significantly more than individuals that did not ($F_{1,41}=5.570$, $p=0.023$).

When the sham+T animals were removed from the analyses (as described above), there was still a significant difference in mean attack number between groups ($F_{2,31}=4.785$, $p=0.015$) (Fig. 1A). A Tukey's post hoc test revealed that the only groups that differed

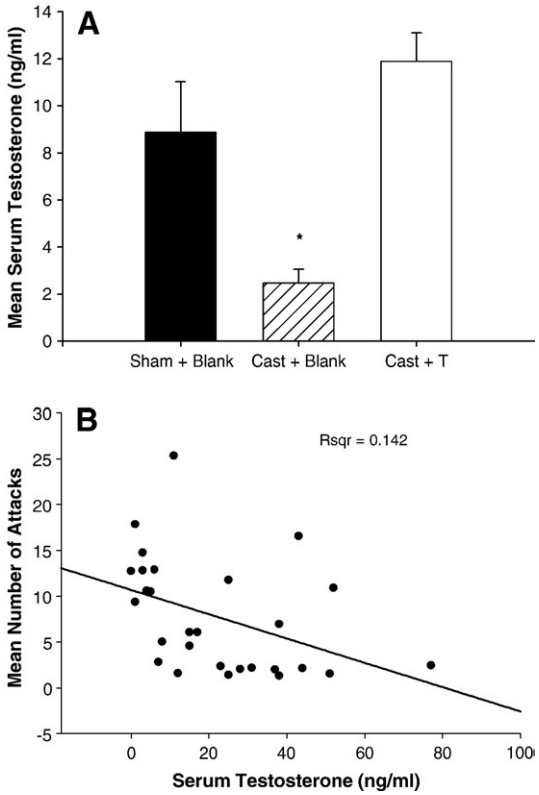

Fig. 2. Mean (±SEM) serum testosterone for castrated or sham-castrated hamsters and implanted with empty (Blank) capsules or testosterone filled (T) capsules (A). Correlation between serum testosterone concentrations and the number of attacks (B). An asterisk (*) denotes statistical significance at $p<0.05$.

significantly from each other were the Cast+Blank and the Cast+T group, with the Cast+T individuals attacking significantly less ($p=0.011$) (Fig. 1A). There was no difference in mean latency to first attack between groups ($F_{2,31}=0.545$, $p>0.05$) (Fig. 1B). Latency was still significantly and negatively correlated to attack number ($R=-0.446$, $p=0.008$).

There was a significant difference in mean serum T levels between groups ($F_{2,\ 16.095}=16.482$, $p<0.001$) and post hoc analysis revealed that Cast+Blank individuals had significantly lower T levels than both Cast+T ($p<0.001$) and Sham+Blank ($p=0.002$) (Fig. 2A). The Serum T levels for Cast+T and Sham+Blank individuals did not differ ($p>0.05$) (Fig. 2A). Serum T levels were negatively correlated with attack number ($R=-0.420$, $p=0.023$) (Fig. 2B). There was no difference between groups for any other behavioral measure (chase (Fig. 1C), AGI, grooming, and climb/escape) ($p>0.05$).

3.2. Experiment 2

Overall, as predicted, short-day hamsters displayed more aggressive behavior than long-day hamsters. Short-day individuals, attacked significantly more compared to long-day individuals ($F_{1\ 27}=7.988$, $p=0.009$) (Fig. 4A). Furthermore, short-day hamsters had significantly shorter latency to first attack ($F_{1,\ 27}=9.474$, $p=0.005$) (Fig. 4B) and chased significantly more than long-day animals ($Z=-2.170$, $p=0.030$) (Fig. 4C). Long-day hamsters, however, performed more anogenital investigations (AGI) than short-day hamsters ($F_{1,\ 27}=6.454$, $p=0.017$) (Fig. 4D). There was no photoperiod effect on grooming ($F_{1,\ 27}=0.007$, $p>0.05$) or escape/climbing behavior ($F_{1,\ 27}=2.224$, $p<0.05$). Additionally there was a significant photoperiod effect on both adrenal ($Z=-2.005$, $p=0.045$) (Fig. 3B) and gonad weights ($Z=-4.815$, $p<0.001$) (Fig. 3A) with short-day hamsters having significantly heavier adrenals and smaller gonads than long-day individuals.

In this experiment there was no effect of DHEA treatment on behavioral measures (chases ($Z=-0.469$, $p>0.05$), numbers of attacks ($F_{1,27}=0.969$, $p>0.05$), the latency to initial attack ($F_{1,27}=0.764$, $p>0.05$), number of grooming bouts ($F_{1,27}=0.058$, $p>0.05$), escape/climbing behavior ($F_{1,27}=0.195$, $p>0.05$) or AGI ($F_{1,27}=0.002$, $p>0.05$)) or morphological measures (adrenal ($Z=0.000$, $p>0.05$) and gonad ($Z=-1.107$, $p>0.05$) weights). There were no significant interactions between DHEA and photoperiod with respect to any behavioral or morphological variables measured ($p<0.05$).

There was no effect of photoperiod on DHEA levels ($F_{1,\ 19}=.336$, $p>0.05$), however, DHEA treatment significantly increased serum DHEA levels ($F_{1,\ 19}=56.492$, $p<0.001$) (Fig. 3C). There was no significant interaction between photoperiod and drug treatment. There was a significant effect of photoperiod on serum T levels. Long-day individuals had significantly higher T levels than short-day individuals ($F_{1,\ 25}=26.352$, $p<0.01$) (Fig. 3D). Further, DHEA treatment also significantly increased circulating T levels ($F_{1,\ 25}=8.122$, $p=0.009$) (Fig. 3D). Once again there was no interaction between photoperiod and treatment with respect to T levels ($F_{1,\ 25}=0.582$, $p>0.05$). There was no significant correlation between DHEA or T levels and any aggressive measures, however, there was a trend for serum T levels to be negatively correlated with attack ($R=-0.357$, $p=0.057$).

4. Discussion

The principal goal of the present study was to investigate the role of the gonadal steroid T and the adrenal steroid DHEA in the mediation of seasonal aggression in Siberian hamsters. In Experiment 1 our hypothesis that T was not required for the maintenance/activation of aggressive behavior in reproductive Siberian hamsters was supported. Specifically, long-day castrated individuals did not exhibit decreased

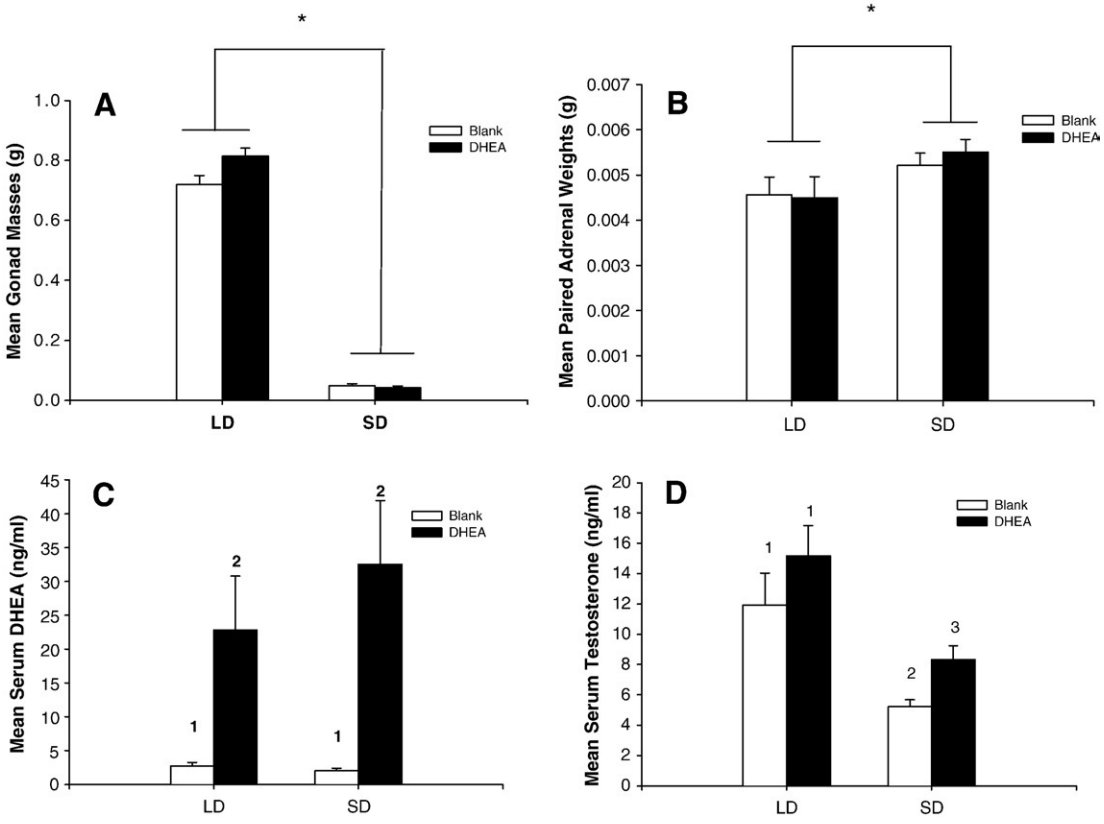

Fig. 3. Mean (±SEM) paired testes masses (A), paired adrenal masses (B) and serum DHEA concentrations (C) in short-day (SD) and long-day (LD) housed animals that received either empty capsules (Blank) or capsules filled with dehydroepiandrosterone (DHEA). An asterisk (*) denotes statistical significance at $p<0.05$.

levels of aggression relative to intact individuals. This is especially interesting because in a closely related hamster species, *Phodopus campbelli*, as well as in Syrian hamsters, males in reproductive condition show decreased territorial aggression toward an intruder when castrated.[5,49]. Further, counter to our initial prediction that exogenous T would not affect aggressive behavior, individuals that received exogenous T were in fact less aggressive than those that received empty capsules. Together, these data suggest, unlike previous studies in other rodents (rats, mice, gerbils, Syrian hamsters)[3–5,7] that individuals in breeding condition do not require gonadal steroids to activate aggressive behavior and perhaps aggression may actually be inversely related to T, as has been previously reported in short-day Siberian hamsters [18]. The effects of exogenous T on aggression were not likely a result of an overall decrease in activity as individuals that received T capsules did not differ in the frequency with which they performed other non-aggression related behaviors (e.g., grooming, AGIs).

Our present finding demonstrating the lack of decreased aggression following castration is intriguing in that it contrasts with a large literature that has reported castration-induced decreases in aggression in reproductively active males in a variety of vertebrate species [3–7,10–12]. Often, castration causes a decrease in territorial aggression whereas exogenous T replacement restores it and may even enhance aggression in intact individuals, even in species that show dissociation between T and aggression during the non-breeding season [3,5,6,14,15,49–51]. Further, there is a well established relationship between anabolic steroids and aggression (e.g.,[52,53]). Much of the previous work on territorial aggression, however, has focused on species that do not display territorial aggression during the non-breeding season, express reduced aggression during the non-breeding season, or maintain similar levels of aggression throughout the year. Studies of Syrian and Siberian hamsters appear unique in that these species exhibit elevated aggression during the non-breeding season. Although castration significantly reduces aggression in reproductive male Syrian hamsters, T is not required to maintain breeding season aggression in Siberian hamsters. Further, aggression appears inversely related to T in adult Siberian hamsters as exogenous T decreases aggression in reproductive males [5]. Perhaps differences in the life histories of these species could account for the differential effects of T on behavior. Unfortunately, little is known about the ecology of these rodents, especially in terms of aggression in the field. It is possible that in Siberian hamsters T facilitates breeding by both enabling males to become physically able to reproduce and reducing aggression enough to allow interaction with female conspecifics. Once again, however, further field work may be required to fully investigate this possibility.

Although enhanced aggression during the non-breeding condition is uncommon, there are several examples of species that maintain territorial aggression during this time, even though T levels are low. Interestingly, circulating levels of the adrenal androgen DHEA correlate with aggressive vocalizations in non-breeding birds (spotted antbirds) [39] and experimentally-induced increases in this hormone increases singing in response to a territorial intrusion (song sparrows) [38]. These studies have naturally led to the investigation of alternative hormonal mediator of non-breeding aggression, including DHEA. Experiment 2 attempted to explore the possibility that DHEA may play a role in the aggression observed in this species. Our results, however, did not support our hypothesis that seasonal changes in

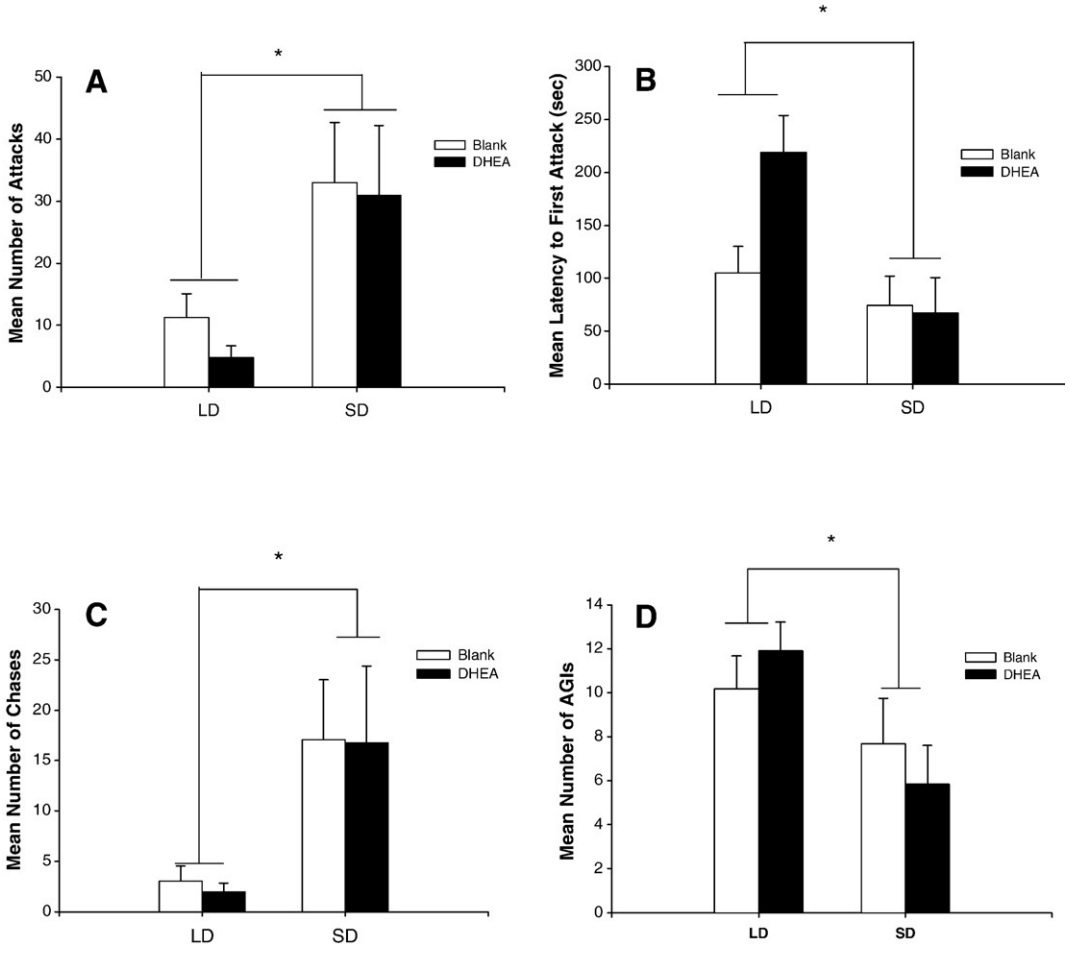

Fig. 4. (A) Mean (±SEM) number of attacks (A), latency to first attack (B), mean number of chases (C) and number of anogenital investigations (AGIs) for short-day (SD) and long-day (LD) housed animals that received either empty capsules (Blank) or capsules filled with dehydroepiandrosterone (DHEA). An asterisk (*) denotes statistical significance at $p<0.05$.

territorial aggression in Siberian hamsters are mediated by circulating levels of dehydroepiandrosterone (DHEA). Short-day hamsters were more aggressive than long-day individuals, but short-day DHEA-treated individuals were not significantly more aggressive than short-day control animals with empty capsules. Long-day controls did not differ from long-day DHEA-treated individuals with respect to any behaviors assayed including aggression. Our results, however, do support previous studies that report an increase in aggression in short-day hamsters [17,18]. Short-day individuals, which had undergone gonadal regression and had significantly lower paired testes masses and T levels compared with long-day males, were more aggressive in every measure (e.g., chases, attacks, latency to first attack) than long-day individuals. Long-day residents also performed significantly more anogenital investigations (AGIs) than did short-day residents. This increase in investigative behavior relative to short-day individuals may be due to the fact that long-day individuals displayed less aggressive behaviors and, therefore, spent more time performing other types of social interactions.

Collectively, our data suggest that circulating levels of DHEA do not increase aggression in Siberian hamsters. There are several other mechanisms, however, by which DHEA may yet prove to be an important regulator of aggression. Although we did not observe an increase in DHEA in short-day animals compared to long-day individuals, DHEA concentrations were similar across photoperiods (as has been reported previously in deer mice (*Peromyscus maniculatus*) [54]). Thus, unlike T, short-day levels of DHEA are not significantly reduced. It is possible, therefore, that the amount of DHEA in circulation does not correlate with aggressive behavior per se, but rather, DHEA serves as a precursor for the conversion to other biologically relevant hormones [24]. This is a distinct possibility because, although DHEA has weak androgenic potency, it is involved in the synthetic pathway that leads to other active sex steroids. For example, DHEA can be converted to androstendione (AE) via the enzyme 17β HSD and subsequently to T via 3β HSD and to 17β-estradiol (E_2) by aromatase [55]. Once converted, these hormones could exert their androgenic or estrogenic effects by binding to androgen or estrogen receptors (i.e. AR, ER respectively) [56,57]. Interestingly, a recent study in Siberian hamsters suggests that short-day housed hamsters show increased numbers of ERα in brain regions that have been found to regulate aggressive behavior [58]. Our DHEA treatment increased circulating DHEA levels beyond what typically occurs in this species. It is possible that a more physiological dose might have resulted in an increase in aggressive behavior. If true, however, this would not likely explain the short-day increases in aggression; we did not observe an increase in DHEA levels in short-day individuals in this study, despite significant increases in aggression. This suggests that heightened circulating levels of DHEA are not required for short-day increases in aggression.

Research on song sparrows suggests aromatization of DHEA to E_2 as a possible mechanism for the mediation of non-breeding aggression [38,59–62]. These data suggest that sex steroids, estrogens in particular, are necessary for the expression of aggressive behavior in the non-breeding season, though plasma sex steroids are non-detectable and castration has no effect on non-breeding aggression[60]. The source of androgen substrate for aromatase in the non-breeding season is hypothesized to be DHEA [59,61,63]. Research investigating seasonal changes in converting enzyme activity (e.g., aromatase, 17β HSD, 3β HSD) in the context of aggressive behavior will likely be fruitful, especially in the light of recent research that suggests that photoperiodic changes in ER may have important behavioral consequences [58,64,65].

Additionally, it is well known that DHEA acts as a neuroactive neurosteroid and is synthesized in the brain of humans and others mammals including hamsters [56]. The present study only addressed the possibility that adrenally derived (and, thus, circulating levels) DHEA affects aggressive behavior. We did not investigate neurally-derived DHEA or (its precursor DHEA-S). DHEA-S is thought to modulate gamma-aminobutyric acid type A ($GABA_A$) receptor functions [66]. More specifically, DHEA-S is acts as a $GABA_A$ antagonist [66]. DHEA-S has also been found to be a positive allosteric modulator of the NMDA receptor. Previous work has suggested that DHEA-S may increase aggressive behavior in mice [66]. Interestingly, circulating levels of DHEA-S are also maintained across photoperiod in Siberian hamsters, however exogenous DHEA-S does not seem to affect aggressive behavior (M.L. Scotti, P. Patel, G.E. Demas unpublished data). It is also possible that adrenal glucocorticoids may be playing a significant role in the mediation of both long-day and short-day aggression in Siberian hamsters. As previously noted, a large body of research has implicated these hormones in the regulation of aggressive behavior in a wide variety of species. We are currently exploring the possibility that glucocorticoids mediate seasonal changes in aggressive behavior in this system.

Collectively, the results of the present suggest that high levels of T are not required to activate aggression in adult hamsters that are in breeding condition and that circulating levels of DHEA do not serve to heighten aggression in this species. The present study does not rule out a potential role for these hormones in seasonal aggression via "downstream" mechanisms (i.e. converting enzymes, receptors); further investigation is required to test these ideas. Regardless of the specific mechanisms, continued study of the regulation of seasonal changes in aggression is important because, at the ultimate level of analysis, increased aggressive behavior in short-day hamsters may confer an evolutionary advantage, allowing survival during resource-poor winter periods. Research in this area may allow better insight into the mechanisms underlying social behaviors in general, as well as the evolution of these mechanisms.

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